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Virulence Genes and Antibiotic Resistance Patterns of *Bacillus cereus* Isolated from Egyptian Milk, Milk Powder, and Ice Cream

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Abstract

One of the most common food poisoning illnesses globally is caused by *Bacillus cereus*, which causes emetic and diarrheal food poisoning. Forty–two (42%) out of 100 samples from raw milk, powdered milk, ice cream, and pasteurized milk were positive for *B. cereus*, with a high prevalence in raw milk at 64%. Three virulence genes (*nheA*, *cytK*, and *hblC* genes) were characterized among 42 *B. cereus* isolates with variable frequencies. Detection of the *nheA* gene showed a high level of 90.4%, followed by the *cytK* gene in percentage at 50%, and the *hblC* gene at 47.6%. All examined strains were resistant to Penicillin, Oxacillin, then Cefixime and Ampicillin (85.7%), followed by Nalidixic acid (73.8%), Sulphamethoxazol-Trimethoprim(61.9%), and Oxytetracycline and Cephalotin (52.3%) whilst sensitive to Gentamicin (75%), followed by Enrofloxacin and Erythromycin (50%). Unfortunately, all examined strains are MDR and show 23 resistance patterns, this represents a real health malice for the people of Egypt. The obtained results demonstrated the rise of pathogenic *B. cereus*, a virulent organism that is multidrug-resistant, in Egypt's retail milk and milk products.

KEYWORDS
Bacillus cereus, Virulence genes, Antibiotic resistance, Dairy products

INTRODUCTION

Bacillus cereus is one of several closely related aerobic spore-forming species that make up the *B. cereus* group. Typically surviving throughout a wide range of temperatures (4-55°C), *B. cereus* is a common Gram-positive spore-forming bacterium that is facultatively anaerobic. Emetic strains have a minimum temperature of 10°C with optimum growth at 30-40°C and are infrequently isolated at 4°C. The highest toxin generation occurs at 20–25°C, with a variety of 10–40°C for toxin production (Felis et al., 2009). Commonly located in low concentrations in dried, raw, or processed food as well as in soil and the environment (Pirhonen et al., 2005). The competitive flora in food is eliminated during cooking, which strongly favors the emergence and multiplication of this bacterium from its spores. The spores of *B. cereus* can withstand dry heat better than moist heat, and they can also withstand oily foods better. Spores can withstand being cooked at or below 100°C (van Asselt and Zwietering, 2006).

The construction of various exogenous enzymes, including hemolysins, proteases, phospholipases, and the capacity to develop biofilms, is essential for *B. cereus* pathogenicity (Tirloni et al., 2020). *B. cereus* can elicit two different types of intoxication. The toxins that produce the two illnesses, emetic toxin, and diarrheal toxin, are very different from one another (enterotoxin). In healthy patients, *B. cereus* illness is typically cured, however extremely deadly cases have been observed (AIFST, 2003).

Enterotoxins such as enterotoxin FM (entFM), enterotoxin T (bceT), hemolysin BL (hbl), non-hemolytic enterotoxin (nhe), and endotoxins such as cry and cytotoxin K (*cytK*) toxins are among the different types of toxins found in *B. cereus* (Mostafa et al., 2022). The three produced pore-forming cytotoxins Hbl, Nhe, and *CytK* are thought to be the main causes of *B. cereus* diarrheal pathogenicity. The three-part toxin known as hemolysin BL consists of two lytic proteins, L2 and L1, and an additional component B that is encrypted by the genes *hblC*, *hblD*, and *hblA*, respectively. The nhe operon, which consists of *nheA*, nheB, and nheC, encodes nhe proteins. In addition to causing necrotic enteritis with a variety of symptoms and bloody diarrhea, *B. cereus* strain isolates that were connected to multiple episodes of food poisoning had *CytK*. *CytK* has also been shown to activate enterotoxin against epithelial cells in the gastrointestinal tract and to form pores in planar lipid bilayers (Senesi and Ghelardi 2010). *B. cereus* is typically known as a food poisoning agent, however different parameters based on the temperature of growth, matrix of feeding, and nutritional accessibility can also alter strains, and all strains have the potential to result in localized wounds and ocular infections in addition to other systemic conditions. (Ehling-Schulz et al., 2019).

This study's objective was to use PCR to isolate and identify *B. cereus*, followed by an antibiotic susceptibility test, to ascertain the forbidden of *B. cereus* in various types of milk and milk products currently in the Mansoura market.

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MATERIALS AND METHODS

Samples collection

Twenty-five separate supermarkets and retail stores in deference areas of Mansoura town, Dakahlia governorate. Egypt was used to acquire a total of 100 random representative samples of raw milk, pasteurized milk, milk powder, and ice cream (small-scale production ice cream) (25 samples, for each)This study was done during January to March, 2023. Each was given a distinct sterile plastic bag, clearly tagged, and maintained in an ice box for transportation to the laboratory of Food Hygiene, Safety and Technology Department, Faculty of Veterinary Medicine, Mansoura University, where it was weighed out for bacteriological analysis. Biosafety measures through sample handling and applying microbiological checks in the laboratory were practical agreeing with the guidelines of WHO (2004).

Isolation and Identification of Bacillus cereus

A sterile blender jar was filled with twenty-five (25g) samples of each product, which were then blended for two minutes at a high speed (10,000–12,000 rpm) with nine times (225 ml) of water containing 0.1% bacteriological peptone before being inoculated at 30°C for eighteen hours. A loopful of the inoculation sample was removed, and the bacteria were subsequently plated on polymyxin-pyrovate-eggyolk-mannitol-bromothymol blue agar (PEMBA) (Oxoid, UK). The *B. cereus* was then cultured at 30°C for 48 h to count the colonies. When evaluated for hemolysis on blood agar, the colonies with blue, turquoise to peacock blue color and copious precipitation surrounding them indicated lecithinase activity on PEMBA. The presumed colonies were recognized by Gram's staining, the existence of spores, the incidence of hemolysis on blood agar, motility test, and biochemical processes, which resulted positive for catalase, gelatin liquefaction, nitrate reduction, egg yolk reaction, citrate utilization, anaerobic utilization of glucose (O/F) tests, and negative for indole test, and growth in nutrient broth with 7% and 10% NaCl.

Molecular identification of B. cereus virulence genes

Complete PCR experiments were run to identify virulence genes (*nheA*, *cytK*, and *hblC* genes). The bacterial genome DNA was extracted using the conventional boiling method from purified suspicious colonies (Zinathi et al., 2015). The PCR master mix (Promega, Madison, USA), 1µL of forward primer, 1µL of reverse primers (Metabion International AG, Germany), 4.5 µL PCR-grade water, and 6 µL of DNA template were used to amplify the extracted DNA in an Applied Biosystem 2720 Thermal Cycler (USA). Table 1, lists the PCR settings and the virulence gene primers that were employed. The PCR products were amplified and then placed on a 1.5% agarose gel, which was stained with 1% ethidium bromide and photographed under UV light. *Bacillus cereus* ATCC 14579 served as a positive control model.

Table 1. Target genes, primer sequences and amplified segments.

Target genes	Primer Sequences	Amplified segments (bp)	Primary denaturation	Amplification (30 cycles)			Final extension	References
				Secondary denaturation	Annealing	Extension		
nheA	F-5′ TACGCTAAGGAGGGGCA ′3 R-5′ GTTTTTATTGCTTCATCGGCT ′3	499	95°C 5 min	94˚C 1 min	51˚C 45 sec	72˚C 2 min	72°C 7 min	Hansen and Hendriksen (2001)
hblC	F-5′ CCTATCAATACTCTCGCAA ′3 R-5′ TTTCCTTTGTTATACGCTGC ′3	695			54˚C 1 min			Ngamwongsatit et al. (2008)
cytK	F-5′ CGACGTCACAAGTTGTAACA ′3 R-5′ CGTGTGTAAATACCCCAGTT ′3	565			58˚C 1 min			

Antibiotic susceptibility testing

On Mueller-Hinton agar (Difco), *B. cereus* strains were tested for sensitivity to 14 commercially available antibiotic discs (Oxoid, Ltd.), using the recommended disc diffusion method (CLSI 2016). The following 14 antibiotics were chosen based on their clinical utility in both human and veterinary medicine: Oxacillin OX (1 µg), Enrofloxacin Nor (5 µg), Cefixime CF (5 µg), Ciprofloxacin CP (5 µg), Ampicillin AM (10 µg), Gentamicin G (10 µg), Penicillin G (10 Units), Erythromycin E (15µg), Sulphamethoxazol SXT (25 µg), Chloramphenicol C (30 µg), Nalidixic acid NA (30 µg), Cephalotin CN (30 µg), Oxytetracycline OT (30 µg), Kanamycin K (30 µg). By calculating the inhibition zone diameter of the antibiotic disc with the strains, the CLSI (2016) recommended standard criteria were used to determine whether the inhibition zone was sensitive, intermediate sensitive, or resistant

RESULTS

Prevalence of B. cereus strains

Among the 100 samples, 42 percent (42) *B. cereus* pathogens were isolated from milk and milk product samples and verified by PCR assay. Considering the sample type, *B. cereus* strains had a high prevalence of 64% (16/25) in raw milk, 48% (12/25) in milk powder, 40% (10/25) in ice cream, and 16% (4/25) in pasteurized milk (Table 2).

Table 2. Detection of B. cereus in milk and milk product

Type of sample	No. of sample	No. (%) of positive isolate
Raw milk	25	16 (64%)
Milk powder	25	12 (48%)
Ice cream	25	10 (40%)
Pasteurized milk	25	4 (16%)
Total	100	42(42%)

Determination of virulence genes in B. cereus strains

Genes involved with pathogenicity were checked for in *B. cereus* isolates (*nheA*, *cytK*, and *hblC* genes). Table 3 provides a summary of the virulence genes found in milk and milk products. A significant percentage of 90.4% (38/42) of all samples had the *nheA* gene identified, followed by the *cytK* gene in percentage at 50% (21/42), and the *hblC* gene at 47.6% (20/42).

The *nheA* gene was present in every isolate from pasteurized milk, 13 isolates from raw milk, 11 isolates from milk powder, and 10 isolates from ice cream. The *cytK* gene was present in ten isolates from raw milk, eight isolates from ice cream, and three isolates from powder. Ten isolates from raw milk, six from ice cream, and four from powder all harbored the *hblC* gene; in contrast, *hblC* gene not detected in isolates from pasteurized milk (Figs. 1, 2 and 3).

Table 3. Prevalence of virulence gene in *B. cereus* strains

Source of strain	*nheA* gene	*cytK* gene	*hblC* gene
Raw milk (16)	13	10	10
Milk powder (12)	11	3	4
Ice cream (10)	10	8	6
Pasteurized milk (4)	4	0	0
Total (%)	38 (90.4%)	21 (50%)	20 (47.6%)

Table 4. Modality of virulence genes of *B. cereus* obtained from milk and milk product.

Strain No.	Serotype	Source of isolated Strain	Resistance pattern	Virulence gene		
				NheA	*cytK*	*hblC*
1	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR/ CIP	+	+	+
2	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ SXT/ KF/ ENR/K	+	+	+
3	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR/K	+	+	+
4	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ SXT/ KF/	+	+	-
5	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ OT/ ENR/	+	-	-
6	*B. cereus*	raw milk	P/ OX/ AM/ NA/ SXT/ OT/ ENR/	+	-	+
7	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ SXT/ KF/	+	-	+
8	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ OT/ KF/ ENR/	+	+	-
9	*B. cereus*	raw milk	P/ OX/ AM/ NA/ SXT/ KF/K	+	-	+
10	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ OT/ ENR/ CIP	+	+	-
11	*B. cereus*	raw milk	P/ OX /CMF/ NA/ SXT/ KF/ ENR/	+	+	-
12	*B. cereus*	raw milk	P/ OX /CMF/ NA/ OT/ KF/ ENR/	+	-	+
13	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ SXT/ KF/	+	+	-
14	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR/	-	+	+
15	*B. cereus*	raw milk	P/ OX/ AM/ CMF/ SXT/ OT/ ENR/	-	+	+
16	*B. cereus*	raw milk	P/ OX/ CMF/ NA/ SXT/ OT/	-	-	+
17	*B. cereus*	milk powder	P/ OX/ CMF/ NA/ SXT/ KF/	+	+	-
18	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ SXT/ OT/	+	+	-
19	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ NA/ OT/ ENR/	+	+	+
20	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ NA/ OT/	+	-	-
21	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ SXT/ OT/ ENR/	+	-	-
22	*B. cereus*	milk powder	P/ OX/ AM/ NA/ SXT/ KF/	+	-	-
23	*B. cereus*	milk powder	P/ OX/ AM/ NA/ OT/ KF/	+	-	-
24	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ NA/ SXT/ ENR/	+	-	-
25	*B. cereus*	milk powder	P/ OX /CMF/ NA/ SXT/ KF/	+	-	-
26	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ NA/ KF/	+	-	+
27	*B. cereus*	milk powder	P/ OX/ AM/ NA/ SXT/ OT/ KF/ ENR/	+	-	+
28	*B. cereus*	milk powder	P/ OX/ AM/ CMF/ SXT/ OT/ ENR/	-	-	+
29	*B. cereus*	ice cream	P/ OX/ CMF/ NA/ SXT/ KF/	+	+	+
30	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ OT/	+	+	+
31	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ SXT/	+	+	+
32	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ OT/ ENR/	+	+	-
33	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ SXT/	+	+	+
34	*B. cereus*	ice cream	P/ OX/ AM/ NA/ SXT/ KF/	+	+	+
35	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ OT/ KF/ ENR/	+	+	+
36	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ SXT/ ENR/	+	+	-
37	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ NA/ KF/	+	-	-
38	*B. cereus*	ice cream	P/ OX/ AM/ CMF/ SXT/ KF/	+	-	-
39	*B. cereus*	pasteurized milk	P/ OX/ AM/ CMF/ NA/ SXT/	+	-	-
40	*B. cereus*	pasteurized milk	P/ OX/ AM/ CMF/ NA/ SXT/	+	-	-
41	*B. cereus*	pasteurized milk	P/ OX/ AM/ CMF/ SXT/ OT/ ENR/	+	-	-
42	*B. cereus*	pasteurized milk	P/ OX/ AM/ CMF/ NA/ OT/	+	-	-

Antimicrobial susceptibility test

By using the disc diffusion method, 14 antimicrobial drugs were tested on a total of 38 isolates of *B. cereus*, and the findings are shown in Table 4. The findings revealed that Gentamicin had the highest activity percentage (75%) followed by Erythromycin and Enrofloxacin (50%) then Kanamycin, Chloramphenicol, and Ciprofloxacin (37.5%), Oxytetracycline (25%), Nalidixic acid, Sulfamethoxazole, and Cephalotin (12.5%), while Penicillin, Oxacillin, Cefixime, and Ampicillin had negative activity percentages. In addition, there were 23 resistance profiles in the isolates that were examined (Table 5). Among this resistance pattern, the most prevalent pattern was P/ OX/ AM/ CMF/ SXT/ KF, P/ OX/ AM/ CMF/ NA/ SXT/ and P/ OX/ AM/ CMF/ SXT/ OT/ ENR represented by 4 (9.5%) strains followed by P/ OX/ CMF/ NA/ SXT/ KF, P/ OX/ AM/ CMF/ NA/ OT, and P/ OX/ AM/ CMF/ NA/ OT/ ENR, displayed by 3 (7.1%) strains. Notably, every isolated strain displaying 23 resistance profiles showed evidence of multidrug resistance (MDR) a minimum of two categories of antibiotics. The presence of the virulence genes *nheA*, *cytK*, and *hblC* in several *B. cereus* strains isolated from milk and milk product samples revealed various patterns of antibacterial resistance as shown in Table 6.

Table 5. Susceptibility of *B. cereus* isolates to antimicrobial agents.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	NO. /42	%	NO. /42	%	NO. 42	%
Penicillin (P)	-	-	-	-	42	100
Oxacillin (OX)	-	-	-	-	42	100
Cefixime (CMF)	-	-	6	14.2	36	85.7
Ampicillin (AM)	-	-	6	14.2	36	85.7
Nalidixic acid (NA)	5	11.9	6	14.2	31	73.8
Sulphamethoxazol-Trimethoprim (SXT)	6	14.2	10	23.8	26	61.9
Oxytetracycline (OT)	10	23.8	10	23.8	22	52.3
Cephalotin (KF)	10	23.8	10	23.8	22	52.3
Enrofloxacin (ENR)	20	47.6	2	4.7	20	47.6
Kanamycin (K)	36	85.7	3	7.1	3	7.1
Ciprofloxacin (CIP)	40	95.2	-	-	2	4.7
Chloramphenicol (C)	42	100	-	-	-	-
Erythromycin (E)	42	100	-	-	-	-
Gentamicin (G)	42	100	-	-	-	-

Table 6. The distribution of antimicrobial resistance profiles among *B. cereus* isolates

Resistant pattern	Number of antibiotics	Number of isolates
1. P/ OX/ AM/ CMF/ SXT/ KF	6	4 (9.5%)
2. P/ OX/ AM/ CMF/ NA/ SXT	6	4 (9.5%)
3. P/ OX/ CMF/ NA/ SXT/ KF	6	3 (7.1%)
4. P/ OX/ AM/ CMF/ NA/ OT	6	3 (7.1%)
5. P/ OX/ AM/ NA/ SXT/ KF	6	2 (4.7%)
6. P/ OX/ AM/ CMF/ NA/ KF	6	2 (4.7%)
7. P/ OX/ AM/ NA/ OT/ KF	6	1 (2.3%)
8. P/ OX/ CMF/ NA/ SXT/ OT	6	1 (2.3%)
9. P/ OX/ AM/ CMF/ SXT/ OT	6	1 (2.3%)
10. P/ OX/ AM/ CMF/ SXT/ OT/ ENR	7	4 (9.5%)
11. P/ OX/ AM/ CMF/ NA/ OT/ ENR	7	3 (7.1%)
12. P/ OX/ AM/ CMF/ OT/ KF/ ENR	7	2 (4.7%)
13. P/ OX/ AM/ CMF/ NA/ SXT/ ENR	7	2 (4.7%)
14. P/ OX/ AM/ NA/ SXT/ OT/ ENR	7	1 (2.3%)
15. P/ OX /CMF/ NA/ SXT/ KF/ ENR	7	1 (2.3%)
16. P/ OX /CMF/ NA/ OT/ KF/ ENR	7	1 (2.3%)
17. P/ OX/ AM/ NA/ SXT/ KF/K	7	1 (2.3%)
18. P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR	8	1 (2.3%)
19. P/ OX/ AM/ NA/ SXT/ OT/ KF/ ENR	8	1 (2.3%)
20. P/ OX/ AM/ CMF/ NA/ OT/ ENR/ CIP	8	1 (2.3%)
21. P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR/ CIP	9	1 (2.3%)
22. P/ OX/ AM/ CMF/ NA/ SXT/ KF/ ENR/K	9	1 (2.3%)
23. P/ OX/ AM/ CMF/ NA/ OT/ KF/ ENR/K	9	1 (2.3%)
Total		42 (100%)

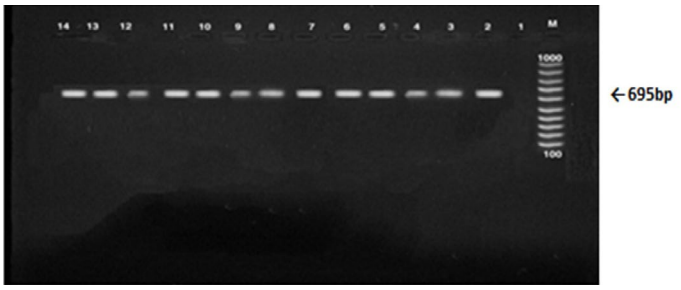

Fig. 1. Representative agarose gel electrophoresis showing amplified PCR products in *B. cereus* isolated of *hblC* gene (695bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *B. cereus*; lanes 3 to 14: positive sample.

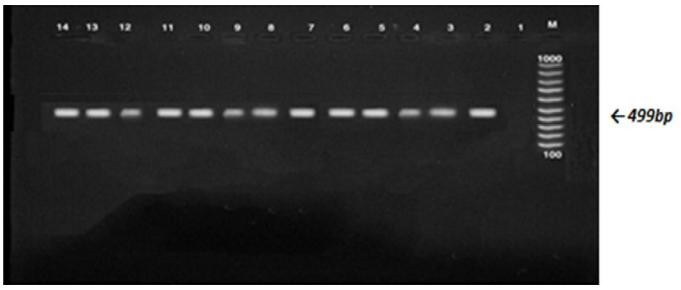

Fig. 2. Representative agarose gel electrophoresis showing amplified PCR products in *B. cereus* isolated of *nheA* gene (499bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *B. cereus*; lanes 3 to 14: positive sample.

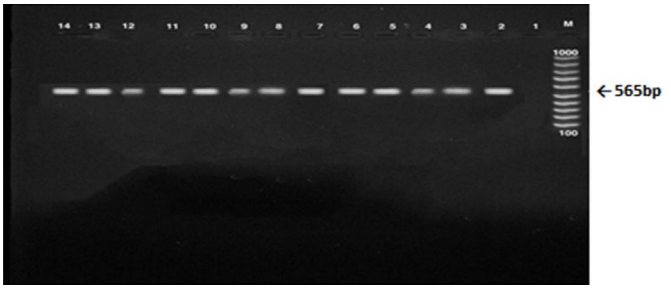

Fig. 3. Representative agarose gel electrophoresis showing amplified PCR products in *B. cereus* isolated of *cytK* gene (565bp). Lane M: 100 bp ladder; lane 1: negative control; lane 2: positive control *B. cereus*; lanes 3 to 14: positive sample.

DISCUSSION

B. cereus poisoning is among the most typical foodborne infections in the globe, which can be caused by tainted milk and milk products (Ceuppens et al., 2013). There is no precise monitoring information on the number of food poisoning brought on by *B. cereus* cases in Egypt. The similarity of the symptoms with those of other foodborne diseases may be the cause of the lack of accurate data (Normanno et al., 2007).

Milk secreted from an uninfected animal's udder is sterile until it is milked, cooled, and/or stored, at which point it becomes contaminated. *B. cereus* grows quickly, especially at high ambient temperatures; additionally, allowing the goods to sit out without refrigeration for a while promotes *B. cereus* replication and thus the release of enterotoxin.

In this research, 42% of milk and milk products contained *B. cereus* overall. The occurrence of *B. cereus* in raw milk (64%) was consistent with Mohamed et al. (2016) and higher than previous studies: 33.3% in China (Chang et al., 2021) and 3.8% in Turkey (Yibar et al., 2017), but less than Osama et al. (2020) 100% in Egypt. Furthermore, the prevalence of milk powder (48%) was lower than in previous studies, which found 52% in India (Kumari and Sarkar, 2014) and 68% in Egypt (Osama et al., 2020), but higher than Wong et al. (1988) 27% in Taiwan and Mohamed et al., (2016) 15% in Egypt.

Also, the presence of *B. cereus* in ice cream (40%) was consistent with earlier studies (Kumari and Sarkar, 2014), while the result is lower than 62.8% in Germany (Messelhäusser et al. 2010) while it is higher than Wong et al. (1988) 35% in Taiwan and Mohamed et al. (2016) 25% in Egypt.

Furthermore, the lowest occurrence (16%) of pasteurized milk in this work is nearly similar to Mohamed et al. (2016) 15% in Egypt, while the result is lower than 41% in Canada (Saleh et al. 2017) and 26% in turkey (Yibar et al., 2017).

Of the 100 samples, milk and milk product samples were used to isolate 41% (41) *B. cereus* strains, which were then verified by PCR assay. Regarding the type of sample, *B. cereus* strains revealed a significant amount of 64% (16/25) in raw milk, followed by 48% (12/25) powder milk, then 40% (10/25) ice cream and 16% (4/25) pasteurized milk.

The virulence genes were discovered in the current study, and the proportion of *nheA*, *cytK*, and *hblC* genes in *B. cereus* was discovered to be 90.4%, 50%, and 47.6% respectively, which is superior to a dairy products inquiry in Turkey (*nheA* 44.8%, *hblC* 17.2 %, *cytK* 0%, Arslan et al., 2014) but were lower than previous studies in China (*nheA* 99%, *hblC* 68%, *cytK* 73%, Gao et al., 2018). Nonetheless, ten (23%) of the isolates in our study had every evaluated virulence gene. However, several isolates tested positive for only a portion of them, which suggests that there may still be risks to the public's health.

Over the world, increasingly pathogenic types of bacteria, most notably *B. cereus*, have developed antibiotic resistance (Kim et al., 2015; Yibar et al., 2017). In this investigation, all identified *B. cereus* strains were resistant to penicillin and oxacillin, followed by cefixime and ampicillin (85.7%), nalidixic acid (73.8%), and sulphamethoxazole-trimethoprim (61.9%) (Fernandes et al., 2014; Amor et al., 2019). The widespread dispersion of -lactamases among *B. cereus* isolates as a result of use in the treatment of urinary tract and respiratory infection and septicemia is the cause of the high resistance to penicillin and oxacillin. It is critical in the treatment of a wide range of illnesses in a variety of animal species where there are few cost-effective alternatives (Kim et al., 2015; Yibar et al., 2017; Amor et al., 2019). In contrast, all strains were sensitive to gentamicin, erythromycin, and chloramphenicol, followed by ciprofloxacin (95.2%), and finally, kanamycin (85.7%) was noticed in previous studies (Shawish and Tarabees, 2017; Yibar et al., 2017). The most clinically significant antibiotics gentamicin, erythromycin, and chloramphenicol have been used to treat *B. cereus*-related gastroenteritis with great success (Savic et al., 2016).

Surprisingly, MDR pathogenic bacteria cause sclerosis in humans and animals, and MDR *B. cereus* strains have been linked to higher morbidity when compared to susceptible bacteria (Jeanand Hsueh, 2011). Unfortunately, MDR was found in all isolates (100%), which was consistent with Mahami et al. (2018) in Ghana and higher than Navaneethan and Effarizah (2021) (35.3%) in Malaysia. The most common pattern of resistance and existence is P/ OX/ AM/ CMF/ SXT/ KF, with 4 (9.5%) strains representing P/ OX/ AM/ CMF/ NA/ SXT. Nonetheless, the discovery of MDR *B. cereus* in milk and milk derivatives emphasizes the importance of food systems as a source of resistance genes. Unfortunately, Egypt has conducted relatively few investigations on the MDR strain of *B. cereus* found in milk and milk products in comparison to other nations.

CONCLUSION

Bacillus cereus strains that are aggressive and multi-drug resistant may be found in milk and milk products, putting Egypt's public health at risk. Thus, thorough hygiene is required to reduce *Bacillus cereus* contamination of milk and milk products.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest concerning this article

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