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Molecular Detection Some *Bacillus spp.* Virulence Factors Genes

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الكشف الجزيئي لبعض لجينات عوامل الضراوة لبكتريا *Bacillus spp.*

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ABSTRACT

Background: In recent years, one of the most pervasive issues regarding global public health is bacterial foodborne diseases

Materials and Methods: A total of 164 different food samples were collected from September 2023 to November 2023 from a range of supermarkets and farmers throughout different locations in Babylon City. Milk products77 (52.70%), meat products19(13%), seeds32(21.90%)and , canned 18(12.32%) . then culture in blood agar, chromogenic agar and not growth on macConkey agar, The virulence gene was amplified via the polymerase chain reaction (PCR).

Results: The prevalence of *Bacillus spp.* , according to the cultured, biochemical, and molecular identification was 17(10.3%). The result of sequencing found the *Bacillus spp*. Isolates belong to the flowing species *B.subtilis* (2*), B.cereus (6), B.thuringiensis(1), B.anthracis (1), B.spizizenii (1) and, Bacillus spp. (6).* The result of molecular detection of virulence gene present *hblA, nheA,* and *entFM* in prevalence 4(23.5),11(64.7) , and 14(82.3) respectively

Conclusion: The isolates of *Bacillus spp.* exhibited a variety of virulence factors correlated with symptoms of diarrhea. As a result, it is imperative to tightly enforce sanitary practices and pay more attention while processing food, adding chemicals only from reliable sources.

Keywords: *Bacillus spp* , Foods , Sequencing, Toxin, Virulence gene

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INTRODUCTION:

A foodborne disease is any illness that is also referred to as a foodborne illness or food poisoning resulting from eating contaminated food. The economic costs of foodborne diseases can severely impact individuals, food companies, and the reputation of countries[1]. Currently, bacterial foodborne illnesses represent one of the most widespread public health challenges globally in recent times [2] , and represent one of the major concerns impacting human health and food safety [3]. Bacteria are responsible for two-thirds of foodborne disease outbreaks, despite the existence of around 250 different foodborne diseases [4].

The diarrheal syndrome is caused by the production of enterotoxin hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe),) following the ingestion of viable cells or spores . The enterotoxins are susceptible to heat, acids, and proteases, it is unlikely that they will cause illness [5]. The emetic disease type is primarily marked by vomiting and nausea, it can occur as quickly as soon as 30 minutes after eating contaminated food [6]. *Bacillus spp.* enterotoxins such as *entFM* exhibit typical characteristics of enterotoxins. Another enterotoxin, *bceT*, has been identified as a diarrheal enterotoxin, displaying biological activities akin to those of diarrheal enterotoxins [7]. The study aimed to investigate the prevalence of some virulence genes among *Bacillus spp.* Isolates from different food samples.

MATERIALS AND METHODS

Samples collection

All total, 164 food specimens were divided into two categories: 19 samples of meat products (such as chicken, beef, and minced meat) and 77 samples of dairy products (including pasteurized milk, raw milk, cheese, and yogurt),32 seeds samples,and 18 canned different foods samples were collected from September 2023 to November 2023 from a range of supermarkets and farmers throughout different locations in Babylon City

Bacterial Identification:

The food samples were measured by weight, distinctly labeled, individually packaged in plastic containers, and and then transported to the laboratory to be suspended the sample in brain heart infusion broth and incubated it at 37°C for 24 hours, followed by culturing on blood agar,

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chromogenic agar and not growth on macConkey agar, we studied the sizes, shapes, textures, and colony organizations of the bacteria. A single colony was picked up, stained with Gram stain, and examined under the light microscope (100x) using oil emersion .

Molecular Identification and detection virulence gene:

The DNA extraction, was done according to manufacturing origin company protocol (Jeneaid ,Taiwan).The polymerase chain reaction mixture for(*16SRNA* **F-AGAGTTTGATCCTGGCTCAG, R-GGTTACCTTGTTACGACTT, 1500bp/ *hbl A* F-GTGCAGATGTTGATGCCGAT,R- ATGCCACTGCG T GGACATAT 320bp/ *nhe A*F-TACGCTAAGGAGGGGCA,R- GTTTTTATTGCTTCATCGGCT , 500bp and *entFM* F-GTT3CGT TCA GGT GCT GGT AC, R-AGC TGGGCCTGTACGTACTT 486bp** in this study was set up for each gene alone in a final volume of 25 µl. The extracted DNA, primers and master mix were vortex and quickly centrifuged to push the material to the tubes' bottom, then placed in a thermocycler polymerase chain reaction. PCR or polymerase chain reaction was employed to amplify the virulence gene [8-10] .

STATISTICAL ANALYSIS

The data analysis by using SPSS V26.

RESULTS AND DISCUSSION:

A total of 164 different food samples were collected from September 2023 to November 2023 from a range of supermarkets and farmers throughout different locations in Babylon City, milk Products77 (52.70%), meat products19(13%), seeds32(21.90%),canned 18(12.32%) the significant difference at P≤0.05 mention in Figure (1), Found the prevalence of of *Bacillus spp.* , according to the cultured, biochemical, and molecular identification was 17(10.3%). The result of sequencing found the *Bacillus spp.* Isolates belong to the flowing species *B.subtilis* (2), *B.cereus (6), B.thuringiensis(1), B.anthracis (1), B.spizizenii (1) and, Bacillus spp. (6).* Figure (2), Table (1). Another researchers isolates 218 *Bacillus spp.* from different food sanples , including *B. cereus* 51%, *B. subtilis*, 22%, *B. amyloliquefaciens*9.1%,, *B. licheniformis* 5.9%[13].

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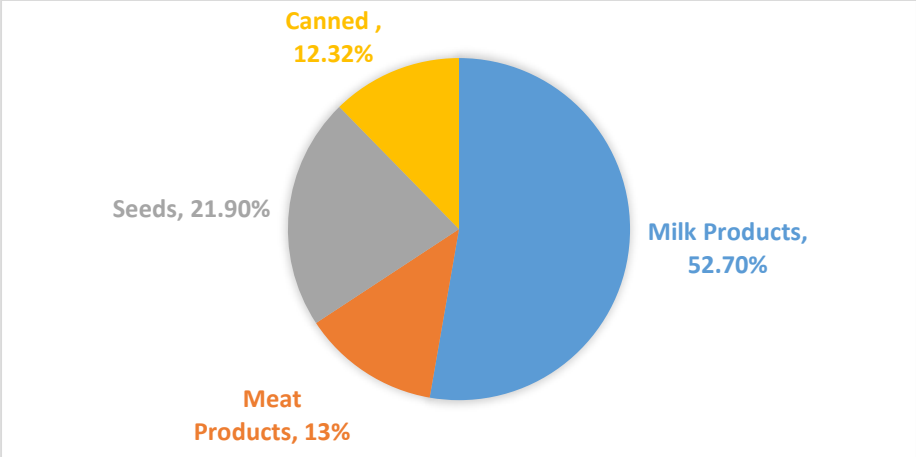

significant difference at P≤0.05.

Figure (1): Distribution food samples

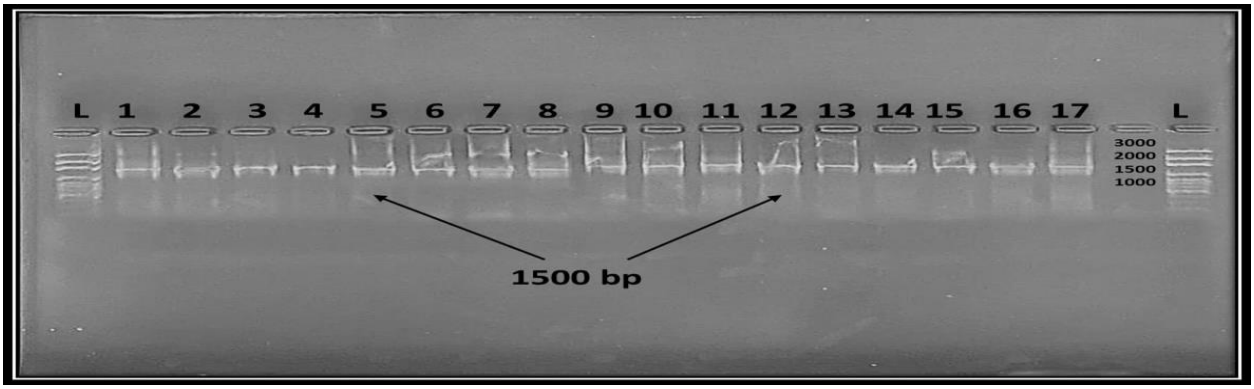

Figure (2): agarose gel electrophoresis staining with goldview, 1.5% was performed at 70 volts for 60 min to detect *16SRNA* PCR products. DNA molecule Lane (L) molecular size marker (1500-bp ladder). demonstrate promising results, and the product is 1500bp in size.

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Table (1): Alignment results of nine local Bacillus reference isolates and retirees from NCBI by *16SRNA* Gene

Local Isolate	Reference of the isolate with highest percentage similarity(%)		
	Accession No.	Similarity (%)	Country
B.subtilis	MH160098.1	87 %	India
B.cereus	EF535596.1	67 %	India
B.thuringiensis	MK509790.1	96 %	Saudi Arabia
B.cereus	KJ948666.1	96 %	China
B.subtilis	MH160098.1	88 %	India
Bacillus spp.	EF601985.1	85 %	India
Bacillus spp.	MH396725.1	83 %	Nigeria
Bacillus spp.	FJ190415.1	71 %	China
Bacillus spp.	KT725621.1	90 %	China
B.cereus	OP602210.1	79 %	Egypt
B.cereus	KF471118.1	69 %	Pakistan
B.cereus	MG430374.1	89 %	India
B.anthracis	KF973315.1	83%	China
B.cereus	MZ197986.1	86 %	Iraq
Bacillus spp.	KR778811.1	90 %	China
B.spizizenii	MN960600.1	93 %	India
Bacillus spp.	MH396750.1	85 %	Nigeria

The result showed the percentage of virulence gene was 4(23.5),11(64.7) ,and 14(82.3) to *hblA, nheA,* and *entFM* , respectively,while not detection *cyt*K in all bacteria isolates Figure (3-8) and Table (2). Other researchers found the percentage was 100%, 9.1%, and 100% to *nhe, hbl,* and *entFM,* respectively among *Bacillus spp.* isolated from food samples[11]. Another researcher found *nheA, hblA,* and *entFM* gene were detected in the *Bacillus spp.* isolates. The *nheA,* and *entFM* enterotoxin genes detected in *B. cereus* , *B. mojavensis* ,and *B. paranthracis* isolates [12].

Because of their hemolytic activity, the majority of *B. cereus* could kill blood cells, indicating their somewhat pathogenicity towards target cells [11]. Also, found the prevalence of *nheA, hblA* and, *entFM* was 55%, 80% and, 33% respectively[13]. Because these virulence genes were spreading across the food and feed chain, they posed a risk to human health. Another study found the percentage of *nhe, hbl gene* isolates was 77.8% and2.0%. among *B. cereus* [14]. Also, it

found the prevalence of toxigenic gene was *nheA* (77%),*hblA* (58%),and *entFM* (84%) distribution among *Bacillus* isolates[15]. However, another researcher's detecting the percentage of toxins gene found the percentage of *nheA* 55% and 80% have *entFM* among *Bacillus spp.* isolates from foods[16].

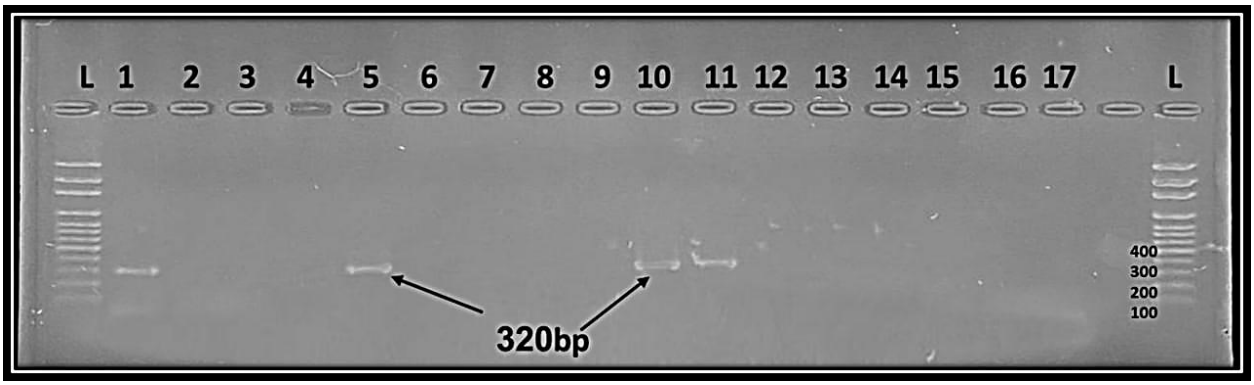

Figure (3): agarose gel electrophoresis staining with goldview, 1.5% was performed at 70 volts for 60 min to detect *hplA* PCR products. DNA molecule Lane (L) molecular size marker (1500-bp ladder). demonstrate promising results, and the product is 320 bp in size.

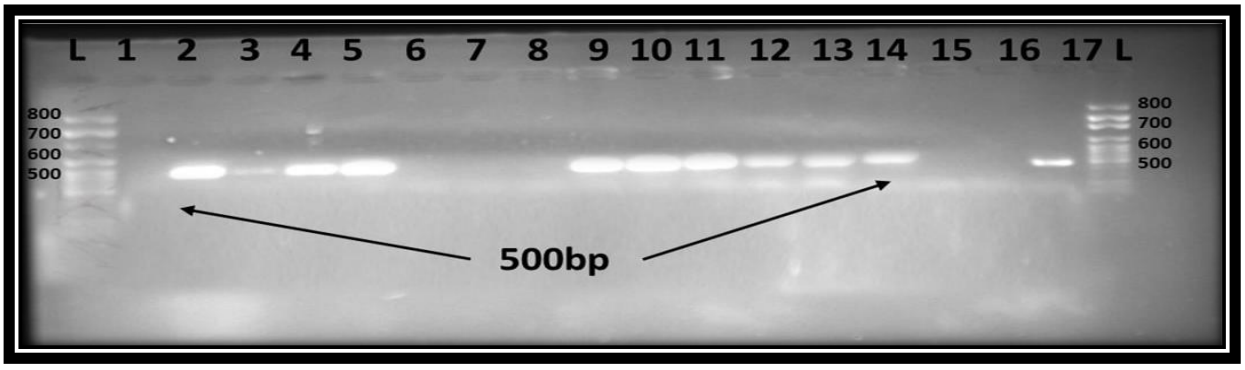

Figure (4): agarose gel electrophoresis staining with goldview, 1.5% was performed at 70 volts for 60 min to detect *nheA* PCR products. DNA molecule Lane (L) molecular size marker (1500-bp ladder). demonstrate promising results, and the product is 500 bp in size.

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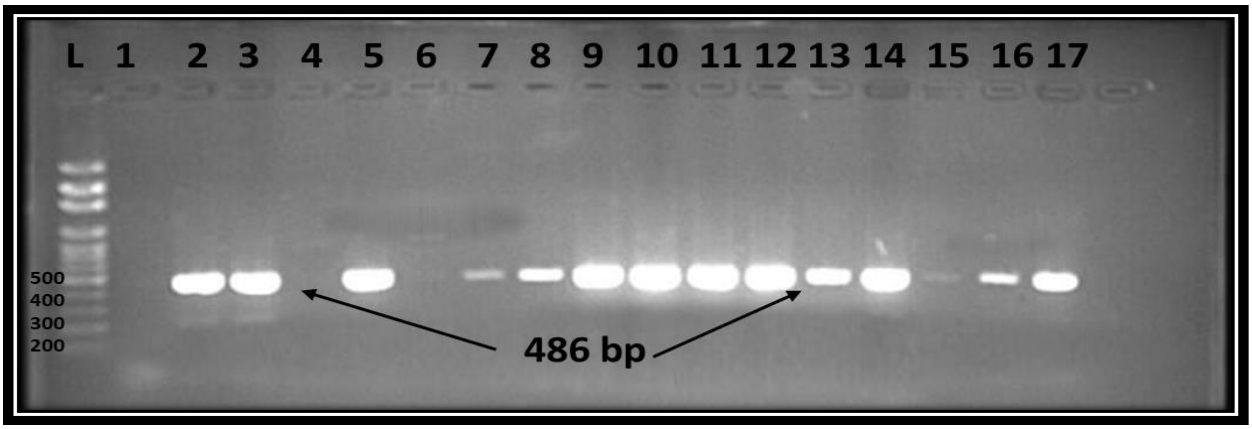

Figure (5): agarose gel electrophoresis staining with goldview, 1.5% was performed at 70 volts for 60 min to detect *entFM* PCR products. DNA molecule Lane (L) molecular size marker (1500-bp ladder). demonstrate promising results, and the product is 486 bp in size.

Table (2) Virulence gene among *Bacillus sp.* isolates

Bacillus spp. / Gene	*hbl*A	*nhe*A	*ent*FM
B.subtilis	+	-	-
B.cereus	-	+	+
B.thuringiensis	-	+	+
B.cereus	-	+	-
B.subtilis	+	+	+
Bacillus spp.	-	-	-
Bacillus spp.	-	-	+
Bacillus spp.	-	-	+
Bacillus spp.	-	+	+
B.cereus	+	+	+
B.cereus	+	+	+
B.cereus	-	+	+
B.anthracis	-	+	+
B.cereus	-	+	+
Bacillus spp.	-	-	+
B.spizizenii	-	-	+
Bacillus sp.	-	+	+
Total NO.(%)	**4(23.5)**	**11(64.7)**	**14(82.3)**

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CONCLUSION

The isolates of *Bacillus spp.* exhibited a variety of virulence factors correlated with symptoms of diarrhea. As a result, it is imperative to tightly enforce sanitary practices and pay more attention while processing food, adding chemicals only from reliable sources.

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

المقدمة: في السنوات الأخيرة، كانت إحدى أكثر القضايا انتشارًا فيما يتعلق بالصحة العامة العالمية هي الأمراض البكتيرية المنقولة عن طريق الأغذية.

طرق العمل: جمعت 164 عينة غذائية مختلفة في الفترة من سبتمبر 2023 إلى نوفمبر 2023 من محلات السوبر ماركت والمزارعين في مواقع مختلفة في مدينة بابل. منتجات الحليب 77 (52.70%)، منتجات اللحوم 19 (13%)، البذور 32 (21.90%)، المعلبات 18 (12.32%). ثم زرعها في أجار الدم، وأجار الكروموجينيك .تم تضخيم جين الفوعة عن طريق تفاعل البوليميراز المتسلسل.

النتائج: وفقا لنتائج التشخيص المزرعي والكيموحيوي والجزيئي بلغت نسبة *Bacillus spp.* 17 (10.3%). نتيجة التسلسل وجدت *Bacillus spp.*تنتمي العزلات إلى الأنواع المتدفقة *B.subtilis* (2)، *B.cereus* (6)، *B.thuringiensis*(1)، *B.anthracis* (1)، *B.spizizenii* (1). نتيجة الكشف الجزيئي لجين الفوعة الموجود *hblA* و*nheA* و*entFM* في *Bacillus spp.* (6). معدل الانتشار 4(23.5)،11(64.7)، و14(82.3) على التوالي.

الاستنتاجات: أظهرت عزلات *Bacillus spp.* مجموعة متنوعة من عوامل الفوعة المرتبطة بأعراض الإسهال. ونتيجة لذلك، من الضروري تطبيق الممارسات الصحية بشكل صارم وإيلاء المزيد من الاهتمام أثناء معالجة الأغذية، وإضافة المواد الكيميائية

الكلمات المفتاحية: *Bacillus spp.* , الأطعمة ,التسلسل، السموم , جين الفوعة