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Risk assessment of *Bacillus cereus* in cooked meat products by using VITEK® 2 and PCR

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ABSTRACT

Bacillus cereus as a food poisoning bacterium threats human health worldwide. Rapid and accurate detection of it in meat products is a demand. Aim of this study was to evaluate risk assessment of *B. cereus* in cooked meat products using VITEK® 2 compact biochemical approach and PCR assay. A total of ninety random samples of cooked meat products represented by luncheon, sausage, and burger (30 of each) were evaluated. *B. cereus* was isolated from meat products with incidence of 7.8 % using VITEK® 2 compact system. PCR screened virulence gene of *B. cereus*. It was found that six samples were positive for *cytk* virulence gene. While, *hbl* virulence gene failed to be detected. Presence of *Bacillus cereus* in meat products is considered a great risk for human health, so accurate and rapid monitoring of meat products using combination of VITEK® 2 (biochemical approach) and PCR identification is recommended for safety of meat products.

1. INTRODUCTION

Food safety is an emerging global issue that impacts world trade and human health. *Bacillus cereus* (*B. cereus*) is one of the most important food poisoning threats all over the world (Zeighami, et al., 2020). Meat and meat products are the most suspected foods to be contaminated with it (Yu et al., 2020). Its food poisoning occurs mainly due to consumption of cooked meat product (Yang et al., 2016), to be a primary dangerous strain as *B. anthracis* (Bhunia, 2018) that may cause death (Naranjo et al., 2011). Add to that, *B. cereus* impacts in forming biofilms that lead to food spoilage and food poisoning (Hussain and Oh, 2017). Moreover, it is considered a very strong preforming strain that hardly removed from food by normal cooking temperature (Azanza and Centeno, 2007) or even low temperature and freeze-thaw (Webb et al., 2019). It also, implicated in other diseases as pneumonia, bacteremia, and endocarditis (Ikeda et al., 2015). It can invade the gastrointestinal tract to cause diarrhea and vomiting (Song et al., 2019). Many virulence factors as Cytotoxic K (CytK) and *hbl* are produced by it and caused diarrhea (Berthold-Pluta et al., 2019).

Although, traditional isolation technique is very effective, it still time consuming, complicated, and labor-intensive (Ding et al., 2017). VITEK® 2 is one of the automated and powerful methods for identification of different strains of bacteria through about 64 miniaturized biochemical tests by using specific cards for certain types of bacteria (Rave et al., 2019). The database of this system is very limited to certain types of bacteria (Costa et al., 2021).

While nuclear detection is a research hotspot (Li et al., 2020) regards to its accuracy, efficiency, and stability (Salman et al., 2020). PCR is one of nuclear techniques for detection of foodborne pathogens (Hansen and Hendriksen, 2001).

Demand for a rapid, accurate, and convenient technique for the detection of food borne pathogens is recommended (Liu et al., 2019). So, this assay aimed for assessment of *Bacillus cereus* from different cooked meat products (luncheon, sausage, and beef burger) using (i) Traditional bacterial isolation, (ii) Automated identification of isolated *Bacillus cereus* strains by using VITEK® 2 compact system, and (iii) multiplex PCR screening for virulence and resistant genes (*hbl and cyt*K genes) from isolated *B. cereus.*

2. MATERIAL AND METHODS

2.1 Collection of samples

A total of 90 random samples of cooked meat products as luncheon, sausage, and beef burger (30 of each) were collected from different retails in El-Menofia governorate. Samples were conveyed to the laboratory following aseptic and safety precautions.

2.2. Preparation of sample:

Collected samples were homogenized in peptone water (1:10), then tenfold serial dilution was prepared.

2.3 Traditional Isolation of Bacillus cereus

Bacillus cereus was isolated according to ISO 7932:2004/Amd 1:2020 on Agar Base-MYP (BC-MYP,

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Biolife) supplemented with polymyxin B sulphate supplement (Code 4240001) and egg yolk emulsion (Code 42111601) then incubated at 37 °C for 24 hours. Suspected colonies (pink colonies) were identified morphologically and then purified for further identification.

2.4. Automated biochemical identification of isolated Bacillus species by using VITEK® 2 compact system.

Isolated strains were examined following instructions of Biomeriux VITEK-2 Compact ref Manual – Ref-414532 (2006).

2.5. PCR identification of virulence genes of B. cereus strains.

PCR was applied to evaluate virulence genes of the isolated *B. cereus*, targeting *hbl* and *cytK* genes.

2.5.1. Extraction of DNA

DNA for each examined strain was extracted according to QIAamp DNA mini kit instructions (Catalogue no.51304).

2.5.2. Preparation of PCR Master Mix

PCR Master Mix was prepared according to Emerald Amp GT PCR master-mix (Takara) Code No. RR310A kit.

2.5.3. Oligonucleotide primers sequences

Gene specific from Metabion (Germany) targeting *hbl* and *cytK* virulence genes of *B. cereus* as in table (1)

Table 1 Oligonucleotide primers sequences) targeting hbl and cytK virulence genes of *B. cereus*.

Primer	Sequence	Amplified product	Reference
hbl	GTA AAT TAI GAT GAI CAA TTTC AGA ATA GGC ATT CAT AGA TT	1091 bp	Ehling-Schulz et al. (2006)
cytK	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421 bp	

2.5.4. the conditions of the primers during PCR

Primers for specific genes were cycled in temperature/time conditions during PCR assay as shown in table (2).

Table 2 Cycling conditions of primers during PCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. cycles	Final extension
hbl	94˚C 5 min.	94˚C 30 sec.	49˚C 1 min.	72˚C 1 min.	35	72˚C 10 min.
cytK	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

2.5.4. Agarose gel electrophoreses.

Agarose gel contained Ethidium bromide was prepared according to Sambrook et al. (1989). The product gel was photographed by a gel documentation system **(Alpha Innotech).** and the data was analyzed through computer software (Microsoft Excel).

3. RESULTS

Results in table (3) revealed incidence of *B. cereus* based on traditional isolation. It was found that incidence of *B. cereus* was 23.3%, 13.3% and 10.0 % in luncheon, sausage, and beef burger, respectively.

Table 3 Incidence of *B. cereus* in the examined cooked meat products samples using traditional technique. (n=30 each)

Meat products	Traditionally positive samples	Percentages%
Luncheon	7	23.3
Sausage	4	13.3
Burger	3	10.0
Total (90)	14	15.6

% according to No. of total samples (90)

Results in table (4) expressed the incidence of *B. cereus* based on VITEK® 2 (biochemical approach). *B. cereus* was biochemically confirmed in four samples (13.3%) from luncheon; two samples (6.7%) from sausage, and one sample (3.3%) from beef burger.

Acceptability of examined meat products was evaluated according to EOS (2005) based on occurrence of *Bacillus cereus* in it and illustrated in table (5). It was found that 92.2% from all examined samples were acceptable to EOS expressed as 86.7%, 93.3%, 96.7% for luncheon, sausage, and beef burger respectively.

Table 4 Incidence of *Bacillus cereus* in examined cooked meat samples using VITEK® 2 biochemical approach. (n=30 each)

Products	VITEK® 2 *B. cereus* positive samples	% of *B. cereus* positive samples
Luncheon	4	13.3
Sausage	2	6.7
Burger	1	3.3
Total	7	7.8

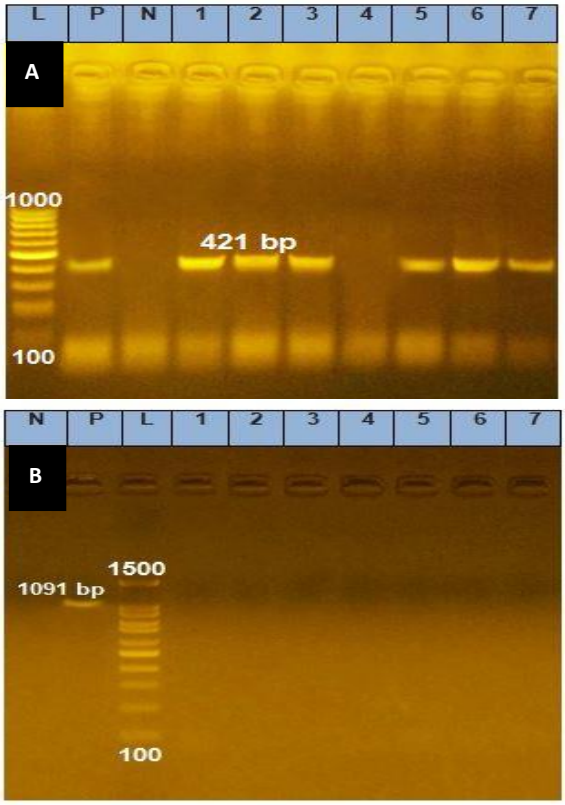
% according to No. of total positive samples (90)

Table 5 Acceptability of the examined meat products according to ES based on their contamination with *B. cereus* (n=30).

Meat products	*EOS* *B. cereus* count /g*	Accepted No	Accepted %	Unaccepted No	Unaccepted %
Luncheon	Free	26	86.7	4	13.3
Sausage	Free	28	93.3	2	6.7
Burger	Free	29	96.7	1	3.3
Total (90)		83	92.2	7	7.8

*Egyptian Standards "ES" (2005).

Agarose gel electrophoresis to PCR products targeting *hblC* and *cytk* virulence genes in isolated *B cereus* strains is shown in photograph (1). *cytk* virulence gene was confirmed in six examined *B. cereus* strains at 421pb, while *hbl* virulence gene failed to be detected at 1091 bp.



Photograph 1 Agarose gel electrophoresis to PCR products targeting *cytk* and *hblC* virulence genes in isolated *B. cereus* strains. (A) *cytk* virulence gene was confirmed in six examined at 421pb in *B. cereus* strains, (B) *hbl* virulence gene failed to be detected at 1091 bp in isolated *Bacillus cereus* strains. N: negative control P: positive control. Lane L: DNA ladder

4. DISCUSSION

Risk assessment of *Bacillus cereus* plays an important role in quality assurance of meat products. *Bacillus cereus* incriminated in many food poisonings owing to its virulence and enterotoxins (Jovanovic et al., 2021).

Bacillus cereus incidence was evaluated in luncheon, sausage, and beef burger cooked meat products traditionally. *Bacillus cereus* was positively detected in examined samples using traditional isolation then confirmed biochemically by biochemical automated approach (VITEK® 2).

These results were nearly agreed with those obtained by Cheng and Quane (2015), who recorded an incidence of 9.53%, and lower than those by Zeighami et al., (2020), who found that *B. cereus* isolates from 12 (15 %) of 80 cooked beef samples, and *Rahnama* et al. (2023) who isolate *B. cereus* from meat and its products with an incidence of 11.87%.

Concerning sausage, these results were lower than those of Fouad et al. (2022), who found a higher incidence of 70% in sausages. Moreover, results of burger were also lower than those of Soleimani et al. (2018), who recorded higher incidences 31.25%.

B. cereus contaminates cooked meat samples along processing chain, and that significantly impacts the quality of final products (Güngör and Gökoġlu 2010). Moreover, storage time and temperature, exposure of food to flies, and poor hygiene measures play a major role in bacterial contamination (Canini et al., 2013).

Using of VITEK 2 Compact® in this approach confirmed isolated strains of *B. cereus*. VITEK 2 Compact® is an automated way for microbial identification provides a trust, accurate, and reproducible results with a lower risk of transcription errors. Moreover, it is a time saving to obtain final results with in about 2-18 hrs, depending on the bacteria's card used (Moehario et al., 2021).

PCR technique has emerged as the fast and reliable technique for the confirmation of enterotoxigenic *B. cereus* and identifying their virulence factors (Ombui et al., 2008).

the dominant virulence *cyt*k gene was detected in 6 out of 7 studied isolates giving product at 421 bp. Meanwhile, *hblC* virulence gene was not detected in all studied isolates. Unlike Fernandes et al. (2014), who detected *hblC* gene in 40% *B. cereus* isolates. Hundred percent of tested *B. cereus* isolates (sausage and beef burger) and 75% of tested luncheon isolates harbored *cyt*k gene close to results reported by Osman et al. (2018) (81.5%). The positive rate of *cytK* was much higher than that observed by Yu et al. (2020) in ready-to-eat food (68%), and those observed by Li et al. (2016). While Zeighami, et al. (2020) used PCR and found that Twenty-six (89.6 %) isolates *B. cereus* carried at least one or more enterotoxin genes.

B. cereus toxins as *Hbl and cytK* that cause diarrhea by rupturing the epithelium have a substantial role in pathogenesis during *B. cereus* gastroenteritis and associated disorders (Etikala et al., 2022). Therefore, it is necessary to ensure that food is free from these toxins.

Cytotoxin K is a pore-forming, necrotic, and hemolytic protein that had been linked to severe food poisoning (Dietrich et al., 2021). Therefore, the presence of this toxin in food poses a great danger to health.

5. CONCLUSION

B. cereus was positively isolated from cooked meat samples and confirmed using VITEK® 2 (biochemical approach). PCR identified virulence/cytotoxic genes in isolated strains. Combination between VITEK® 2 (biochemical approach) and PCR improve risk assessment in meat products. So, reliable molecular monitoring of pathogenic *B. cereus* is strongly recommended for the routine food examination to safe human health.

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