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Molecular detection of antibiotic resistant *bla* gene in *B. cereus* isolated from meat products

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ABSTRACT

In this study the incidence of *Bacillus cereus* (*B. cereus*) and antibiotic sensitivity test was undertaken. A total of one hundred random samples of meat products of rice kofta, kobeba, chicken pane and chicken nuggets (25 of each) were collected from different supermarkets at different times in Menofiea and Kalyobia governorates, Egypt and tested for occurrence of Bacillus group. *Bacillus cereus* were detected in 24%, 12%, 20% and 10% of kobiba–shami, rice kofta, chicken pane and chicken nuggets, respectively. *Bacillus mycoids* failed to be isolated from kobiba–shami and isolated from 4%, 16% and 4% of rice kofta, chicken pane and chicken nuggets, respectively. Also, *B. thuringenesis* failed to be isolated from kobiba–shami and rice kofta while it isolated from 8% of both chicken pane and chicken nuggets. Fourteen strains of *B. cereus* were examined for antimicrobial susceptibility testing. The foremost common drug resistance was to penicillin, amoxicillin and amoxicillin + clavulanic (100% for each). On the other hand, *B. cereus* was completely prone to vancomycin and gentamycin (100% for each).Using PCR, all tested *B. cereus* isolates harbored *bla* gene

1. INTRODUCTION

B. cereus is the most important foodborne pathogen in the *B. cereus* group and has been associated with food-poisoning illnesses in humans and other kinds of clinical infections (Bottone, 2010).

B. cereus produces two types of toxins-emetic (vomiting) and diarrhoeal-causing two types of illness. The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine (Ehling-Schulz et al., 2006).

Meat and meat products are a suitable media for growth because they are high in moisture, high in nitrogenous compounds (amino acids, peptides, and proteins) and abundantly supplied with minerals and accessory growth factors. Furthermore, they have some fermentable carbohydrates, usually glycogen and keep favorable pH for growth of most microorganisms (Galvaz et al., 2010).

B. cereus give purple color by Gram stain, motile (flagellated), sporulated, rod shaped bacterium which belongs to the Bacillus genus (Montville and Matthews 2005).

Program identification of *Bacillus cereus* is commonly involved of isolation on selective media, showing of motility, hemolysis pattern on blood agar, and production of acid from glucose fermentation (Stenfors Arnesen et al., 2008).

The most effective method for treating bacterial infection was antibiotic treatment, containing those produced by *Bacillus cereus*; however, the appearance of antibiotic-resistant strains due to the wide usage of antibiotics, including those resistant to many antibiotics, that may make antibiotic treatment to be unsuccessful (Friedman et al., 2016). So, knowing the antibiotic resistance of *Bacillus cereus* is vital for using the drug of choice for bacterial treatment.

Multiple drug resistant isolates of *Bacillus cereus* because of production of beta-lactamase have a significant threat to Public Health. Beta-lactamases, being one amongst the potential virulence factors make these strains resistant to penicillin, ampicillin, and even to third generation cephalosporin (Cormian et al., 1998).

PCR has become one of the most important molecular diagnostic methods for detection of foodborne pathogens and is considered to be a valuable alternative to the culture-based detection techniques due to its speed, limit of detection (LOD), sensitivity and specificity (Maurer, 2011; Rodríguez-Lázaro et al., 2013). So, the present study was designed for molecular detection of antibiotic resistant *bla* gene in *B. cereus* isolated from meat products.

2. MATERIAL AND METHODS

2.1. Collection of samples

One Hundred random samples of meat products of rice kofta, kobeba, chicken pane and chicken nuggets (25 of each) were collected to be examined for occurrence of *B. cereus.* Each sample was positioned in sterile plastic bags, transported to the lab on icebox and reserved under 4°C.

2.2. Preparation of samples

According to (APHA, 2002), aseptically 10 grams of every sample was put into 90 ml of 0.1% peptone water in a sterile plastic bag, and at that time mixed in a Stomacher

400 Lab Blender (Seward Medical, London, UK) for 30 seconds.

2.3. Isolation of B. cereus

Isolation was conducted following (Shinagawa, 1990); briefly, samples were inoculated into brain heart infusion broth (BHIB) containing polymyxin (100 units/ml). The broth tubes were incubated at 37°C for 24-48 hours. Later a loopful taken from enrichment broth and put on PEMBA (Polymyxin pyruvate egg-yolk mannitol–bromothymol blue agar plates and incubated at 37°C for 24 hr. *Bacillus cereus* grow as crenate, fimbriate, or slightly rhizoid colonies up to 5 mm diameter, turquoise to peacock blue in color with flat ground glass surface, and surrounded by a precipitate from hydrolyzed egg yolk.

2.4. Identification of Bacillus cereus:

B. cereus isolates were subjected for various biochemical tests like catalase test, nitrate reduction test, motility test, lecithinase activity, modified Voges-Proskauer (VP) test, haemolysis on 5% sheep blood agar and various sugar (Inositol, dulcitol, fructose, dextrose, sucrose, mannitol and salicin) fermentation test were conducted. The suspected isolates were identified morphologically and biochemically according to Koneman et al. (1992). The presumptive isolates showing positive catalase test, Voges-Proskauer test, positive nitrate reduction test, positive for anaerobic utilization of sugars by *Bacillus cereus* and produce β-haemolytic.

2.5 Antibiotic sensitivity Testing:

Bacillus cereus isolates were tested to their sensitivity to 9 antibiotics (Penicillin (10 µg), Oxacillin (10 µg), Oxacillin+ Clavulanic acid (30 µg), Co trimoxazole (25 µg), Vancomycin (30 µg), Erythromycin (15 µg), Gentamicin (10 µg),Ciprofloxacin (5 µg) and Kanamycin (30 µg)) in line with the standard disc diffusion method (The Clinical and Laboratory Standards Institute (CLSI), 2010 and Yu et al., 2019). Isolates were put on the plate of Mueller- Hinton agar and incubated for 16–18 h at 37°C. The colony afterward was chosen and suspended in 0.85% physiological saline to 0.5 McFarland standards and placed on the surface of a Mueller-Hinton agar plate. After the inoculum was dried, the antibiotic discs were placed on the surface of the plates. The plates of Mueller-Hinton agar were incubated for 16–18 h at 35 ± 2°C, and the zone of inhibition was measured. The isolates were categorized as susceptible (S), intermediate (I), or resistant (R) in line with Clinical and Laboratory Standards Institute (CLSI) guidelines and also measuring the different zones of inhibition and interpreted as said by the interpretation of zone diameter measures for *staphylococcus aureus* (CLSI 2010).

2.6. Detection of bla gene by Polymerase Chain Reaction

2.6.1. DNA extraction:

Genomic DNA extraction and purification was done using QIAamp DNA Mini Kit. PCR was done to detect *bla* gene. A positive reference strain of *Bacillus cereus* ATCC 14579 and sterile MilliQ water as a negative control was used in PCR analysis (Ehling-Schulz et al., 2006; Das et al., 2013).

Table 1 Provides details about the primer used.

Gene	Sequence of primer (5'-3')	amplified product length	Reference
Bla	F 5'CATTGCAAGTTGAAGCGAAA3'	680 bp	Chen et al., 2004
	R 5'TGTCCCGTAACTTCCAGCTC3'		

Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit as shown in table (2)

Table 2 Preparation of PCR Master Mix

Component	Volume/reaction
Emerald Amp GT PCR master mix (2× premix)	12.5 µl
PCR grade water	4.5 µl
Forward primer (20 pmol)	1 µl
Reverse primer (20 pmol)	1 µl
Template DNA	6 µl
Total	25 µl

Table 3 Temperature and time conditions of the primers during PCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Bla	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Gel electrophoresis was used to analyze PCR fragments.

3. RESULTS

It was evident from the result recorded in table (4) that, *B. cereus* was present in kobeba–shami, rice kofta, chicken pane and chicken nuggets at percentage of 24%, 12%, 20% and 10% of respectively, *Bacillus mycoids* failed to be isolated from kobiba–shami but isolated from rice kofta, chicken pane and chicken nuggets at percentage of 4%, 16 and 4% of respectively . *Bacillus thuringenesis* is failed to be isolated from kobeba–shami and rice kofta while it isolated from both 8% of chicken pane and chicken nuggets.

As shown in table (5), Disc diffusion susceptibility testing revealed that *B. cereus* isolated strains from meat products were susceptible to Vancomycin 30µg (100%), Gentamycin 10µg (100%) and Ciprofloxacin 5µg (78.5%) and highly resistant to Penicillin 10µg (100%), Amoxicillin 10µg (100%) and Amoxicillin + clavulanic acid 30 µg (100%).

Photo (1) declared the results of testing of strains of *B. cereus* isolated from different meat products using specific primer for *bla* antibiotics resistance gene. *bla* gene was found in 100% of isolates of *B. cereus.*

Table 4 Incidence of *Bacillus serotypes* isolated from the examined meat products (n=25)

Products	*B. cereus*		*B. mycoids*		*B. thuringenesis*	
	No	%	No	%	No	%
Kobiba–shami	6	24	0	0	0	0
Rice kofta	3	12	1	4	0	0
Chicken pane	5	20	4	16	2	8
Chicken nuggets	4	16	1	4	2	8

Table 5 Antibiotics sensitivity test of *B. cereus* isolated from examined samples by disc diffusion method (n=14)

Antimicrobial agents	Sensitive		intermediate		Resistant	
	No.	%	No.	%	No.	%
Penicillin 10 µg	0	0	0	0	14	100
Amoxicillin 10 µg	0	0	0	0	14	100
Amoxicillin + clavulanic acid 30 µg	0	0	0	0	14	100
Cotrimoxazole 25 µg	2	14.2	5	35.7	7	50
Vancomycin 30 µg	14	100	0	0	0	0
Erythromycin 15 µg	6	43	2	14	6	43
Gentamycin 10 µg	14	100	0	0	0	0
Ciprofloxacin 5 µg	11	78.5	3	21.5	0	0
Kanamycin 30 µg	6	43	5	35.5	3	21.5

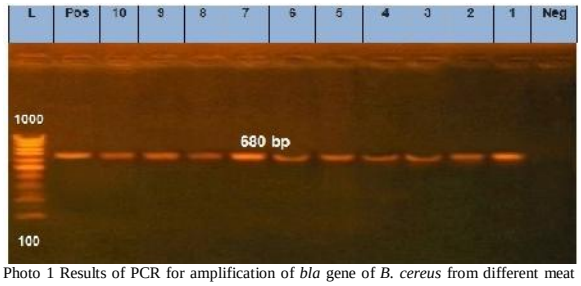

Photo 1 Results of PCR for amplification of *bla* gene of *B. cereus* from different meat and chicken products Neg: negative control, Pos: positive control, Lane L: 100-1000 bp DNA ladder. Lane 1-10: positive samples at 680 bp

4. DISCUSSION

Strains of food-borne bacterial pathogens that are resistant to a variety of antibiotics have become a major health concern (Kiessling et al., 2002; Roy et al., 2007). Results in table (3) revealed that *B. cereus* was the predominant isolated serotype from the examined samples of kobeba–shami, rice kofta, chicken pane and chicken nuggets, respectively. On the other hand, *B. mycoids* failed to be isolated from kobeba–shami and isolated from rice kofta, chicken pane and chicken nuggets, respectively. *Bacillus thuringenesis* is failed to be isolated from kobeba–shami and rice kofta while it isolated from 8% both chicken pane and chicken nuggets. Abd El Wahab et al. (2018) isolated *B. cereus* in percentages of 60, 52 %from rice kofta and kobeba, respectively. Esraa (2018) found that the incidence of *B. cereus* in chicken nuggets was 48%, meanwhile, Dowidare (2009) isolated *B. cereus* from 12% of examined kobeba.

As shown in table (4), 14 strains of *B. cereus* were tested for antimicrobial susceptibility testing. The most common drug resistance was to penicillin, amoxicillin and amoxicillin+clavulanic. Similar results were obtained by Abd El Tawab et al. (2019) for amoxicillin + clavulanic (100%), Fielder et al. (2019) for penicillin (100%) and amoxicillin +clavulanic (99.3%), Yu et al. (2020) for penicillin (100%) and amoxicillin + clavulanic (97.83%) and Guven et al. (2006), who found that *B. cereus* isolates from meat and meat products showed a high resistance to amoxicillin.

On the other hand, *B. cereus* was completely susceptible to vancomycin and gentamycin (100% for each). Similar results were obtained by Shawish and Tarabees (2017) for vancomycin (100%) and Dikbas (2010) (100%) and Yu et al. (2020) (96.47%) for gentamicin. Also *B. cereus* was susceptible to ciprofloxacin, erythromycin, co-trimoxazole and kanamycin. Nearly similar result obtained by Fielder et al. (2019) (99.3%) and Yu et al. (2020) (78.8%) for ciprofloxacin and (76.36%) and Dikbas 2010 (67.5%) for kanamycin.

In our study all tested *B. cereus* isolates harbored *bla* gene which agreed with Abd El Tawab et al. (2019) (100%).

These variations in the results were attributed to the quality of raw materials and the hygienic state during preparation and processing of the product.

The high frequency of isolation of *B. cereus* from meat products may be attributed to processing of minced meat also additives and spices that added to this products, which can increase the number of Bacillus spores. Therefore, it is important to use additives from a trustful source during processing of raw meat and test these additives regularly for the presence of bacillus spore (Shawish and Tarabees, 2017).

Food-poisoning by *B. cereus* can be produced by either infection or intoxication, which leads to a diarrheal or an emetic type of disease, respectively. The diarrheal syndrome is caused by enterotoxins produced by vegetative cells during the growth in the small intestine after consumption of contaminated foods (Schoeni & Wong, 2005). The emetic syndrome is associated with the production of the cereulide toxin in foods before consumption (Ehling-Schulz et al., 2004; Stenfors Arnesen et al., 2008). Beta-lactamases, being one amongst the potential virulence factors make these strains resistant to penicillin, ampicillin, and even to third generation cephalosporin (Cormian et al., 1998)

5. CONCLUSION

We can conclude that *B. cereus* was the predominant isolated serotype of Bacillus group, chicken panne and nugets are the most contaminated products and all tested *B. cereus* strains were harbored the antibiotic resistant and *bla gene.*

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