Molecular studies on toxigenic strains of *bacillus cereus* isolated from some meat products.

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A B S T R A C T

A total of 120 random samples of meat products including luncheon, sausage, minced meat and beef burger (30 from each) were collected from different supermarkets in Gharbia Governorate. The collected samples were transferred directly to the laboratory to be examined bacteriologically for the detection of *B. cereus*. All samples were cultured at 37°C for 24 hours aerobically on selective media (PEMBA) for isolation and purification and on sheep blood agar to observe the hemolysis properties. The results revealed that the incidence of *B. cereus* was 20%, 36.37%, 40% and 56.67% in luncheon, beef burger, sausage and Minced meat, respectively. The suspected colonies were examined for their colonial morphology, microscopical examination, and biochemical reactions.VITEK2 BCL Card was used for more identification of *B. cereus* strains. The public health hazards of the isolated strains were discussed.

Keywords: meat products, *B. cereus*, identification.

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1. INTRODUCTION

B. cereus is a Gram-positive, motile (flagellated), spore-forming, rod shaped bacterium that belongs to the Bacillus genus (Montville and Matthews 2005). A common feature between species within the Bacillus genus is their ability to produce endospores (spores). Spores are dormant structures formed when the bacteria are exposed to dry, low-nutrient or generally adverse environmental conditions (Nicholson et al., 2000). Ready to eat meat products are highly demanded due to their high biological value, reasonable price, agreeable taste and ease of serving (Soliman, 1999). Food-borne diseases, the major cause of morbidity and mortality, are reported to be serious threats to public health all over the world. Among the organisms responsible for causing foodborne diseases, *Bacillus cereus* has emerged as major foodborne pathogen during the last few decades (Jay, 2005). It can cause two types of food poisoning, known as the emetic and the diarrheal types (Granum and Lund, 1997). *B. cereus* produces several virulence factors including toxins and enzymes, which are considered the most important factors. Among these factors are hemolysin, protease and lecithinase (Wu et al., 2008). The aim of this work is isolation and identification of *B. cereus* strains from some meat products.

2. MATERIAL AND METHODS

2.1. Samples collection

A total 120 random samples of meat products (luncheon, sausage, minced meat and beef burger 30 from each) were collected from different supermarkets at Gharbia Governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions.

2.2. Bacteriological examination of B. cereus

Accurately 10 g from each sample were weighed aseptically and homogenized in 30 ml of Brain Heart infusion broth (BHIB) and incubated for 18 hours at 37°C. A loopful of inoculated Brain Heart infusion broth was streaked onto selective media (PEMBA) and incubated aerobically for 24 hours at 37°C. The suspected colonies were inoculated for 24 hours at 37°C onto sheep blood agar for hemolysis properties. The plates containing characteristic colonies of *B. cereus* were selected and the Gram staining test was performed. Each colony showing typical colonial appearance was subjected to biochemical identification and examined for Catalase test, Voges–Proskauer test, Nutrient gelatin tubes, Simmon's citrate agar and Starch agar. Nitrate broth tubes, Indol production test and Urease test.

2.3. Identification of B. cereus using VITEK2 BCL Card.

Bacterial suspensions were prepared in 3.0 mL of sterile saline and adjusted to a McFarland standard of 1.80-2.20 using the VITEK2 DensiChek (bioMe´rieux). BCL cards were filled automatically in the VITEK vacuum chamber, sealed, incubated at 35.5^0C and read automatically every 15 min for 14 hours. Data were analyzed automatically using the VITEK2 database.

3. RESULTS

3.1. Identification of the isolated B. cereus strains

B. cereus on selective media (PEMBA) appeared as blue, turquoise to peacock blue and surrounded by a zone of egg yolk precipitation while it showed Beta hemolysis on blood agar medium. All isolates showed similar pattern of reaction despite of the source of isolation. Catalase, Voges–Proskauer, Citrate utilization, Gelatin hydrolysis, Starch liquification and Nitrate reduction tests showed positive results while indole and Urease showed negative results.

3.2. Prevalence of B. cereus in meat products

The incidence of *B. cereus* was 20%, 36.37%, 56.67% and 40% in luncheon, beef burger, Minced meat and sausage, respectively (Table 1)

3.3. VITEK2 BCL Card.

VITEK2 BCL card provides a major advance in the reliable identification of *B. cereus* (table 3).

Table (1) Prevalence of isolated *B. cereus* from meat products

No. of samples	Cultural examination		% of positive samples	% of negative samples
	Positive	negative		
120	46	74	38.33 %	61.67%

Table (2) Prevalence of *B. cereus* recovered from meat each product

Type of samples	Number of samples	Number of positive samples	% of positive samples
Luncheon	30	6	20%
Beef Burger	30	11	36.67%
Minced meat	30	17	56.67%
Sausage	30	12	40%

Table (3) Result of VITEK2 BCL Card

Identification Information	Card:	BCL	Lot Number: 239316610	Expires:	Aug 15, 2015 13:00 CDT
	Completed:	Apr 1, 2015 23:34 CDT	Status: Final	Analysis Time:	14.25 hours
Selected Organism	Bionumber: 4363500550646651	Bacillus Cereus		Confidence:	Good identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					

Biochemical Details																
1	BXYL	(-)	3	LysA	(-)	4	AspA	+	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	-	10	PyrA	+	11	AGAL	+	12	AlaA	+	13	TyrA	+	14	BNAG	-
15	APPA	+	18	CDEX	-	19	dGAL	+	21	GLYG	-	22	INO	-	24	MdG	-
25	ELLM	-	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	-	31	dMAN	+
32	dMNE	+	34	dMLZ	-	36	NAG	+	37	PLE	-	39	IRHA	-	41	BGLU	-
43	BMAN	-	44	PHC	+	45	PVATE	(+)	46	AGLU	-	47	dTAG	-	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	+
60	OLD	+	61	ESC	(-)	62	TTZ	+	63	POLYB_R	+						

4. DISCUSSION

The over-all prevalence of *B. cereus* in all meat products was 38.33 %, which nearly agreed with some other studies such as 42% by Agarwal et al. (1997) and 40% by Rather et al. (2011). These results are higher than the percentages obtained by other studies such as 18.3% by Konuma et al. (1988), 28% by Schlegelova et al. (2003) and 30.85% by Tewari et al. (2012). However, these results are lower than the percentages obtained by other studies such as 48% by Giffel et al. (1996) and 56.3% by Bedi (2004). The results presented in table (1) revealed that the incidence of *B. cereus* was 20% in the examined luncheon samples. This result agreed with that reported by Samir et al. (2012) and Atia (2014). These results considered low when compared with that reported by 70% El–Ghamry (2004) and 35% Ghanaym (2014). However, this incidence was higher when compared with that recorded with Hamouda (2005) who found that the incidence was 16%.

The incidence of *B. cereus* was 36.67% in the examined beef burger samples. This result was nearly similar to that obtained by Ghanaym (2014) whose result was 35%.This result considered low when compared with that reported by other studies as 65% by Heikal et al. (2006**)** and 92% by El-Mossalami et al. (2008). *B. cereus* was isolated from minced meat samples by 56.67%.This result was nearly similar to that obtained by Hamouda (2005). However, this incidence was higher when compared with that recorded with other studies as 31.4% by Torky (1995) and 22% by El-said (2005). This result considered low when compared with that reported by other studies as 98% by Saleh et al. (1993) and 72% by Abu-Elnaga (2003). *B. cereus* was isolated from sausage samples with a percentage of (40%).This result agreed with that reported by Torky (1995) and Ghanaym (2014). However, this incidence was higher when compared with that recorded with other studies as 28% by Hefnawy et al. (1984) and 30% by Eid et al. (2008). This result considered low when compared with that reported by other studies as 84% by Hamouda (2005) and 70% by Heikal et al. (2006). The results of biochemical tests were in agreement with Saleh et al. (1993) and Enan et al. (2012). VITEK2 BCL Card is advanced and highly sensitive method for identification of *B. cereus.* And this reported by Halket et al. (2010).

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