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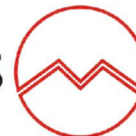

Molecular detection of some toxigenic bacteria isolated from meat and its products in Kaliobia, Egypt

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Abstract: The present study was performed on 250 random samples of fresh meat and meat products viz: Beef burger, kofta; minced meat and sausage (50 for each), were collected from different shops at Kaliobia Governorate, Egypt, for detection the prevalence of some toxigenic food-borne bacteriain these samples, beside the phenotypic characterization and detection of some virulence genes in them. Bacteriological examination of the collected samples resulted in, isolation of 87 (34.8%) isolates of *S. aureus* isolates (41/16.4%); *E. coli* (25 /10.0%) and *B. cereus* (21/8.4%) were recovered from 250 samples. Twenty five *E. coli* isolates were recovered from minced meat samples (7/14%), kofta (6/12.0%); sausage (5/10.0%); fresh meat (4/8.0%) and beef burger samples (3/6.0%). Seven serogroups (O55: H7; O_{111}: H_4; O_{125}: H_{18}; O126: H7; O128: H27; O142: H2and O158: H2) were detected in 25 isolated *E. coli* strains. A total of 41 *S. aureus* isolates were isolated from kofta samples (12 / 24.0%), minced meat (9/ 18.0%), sausage, fresh meat (8/16.0% for each) and beef burger samples (4/8.0%). Twenty one *B. cereus* strains were isolated from kofta (7/14.0%), sausage (6/12.0%); minced meat (4/8.0%); beef burger (3/6.0%) and fresh meat samples (1/2.0%). The PCR results for *E. coli* strains showed that, *stx*2 virulence gene was detected in one strain and *vt2e* virulence gene was detected in two out of 6 studied strains, but *stx*1 virulence gene was failed to be detected in all studied strains. Meanwhile, PCR results for *S. aureus* strains showed that, enterotoxin *seb* virulence gene was detected in one strain and enterotoxin *sed* virulence gene was detected in 4 out of 5 studied strains, but enterotoxins *sea*; *sec* and *see* virulence genes were failed to be detected in all studied strains. In addition, the results for *B. cereus* strains cleared that, *nhe; cyt* K and *ces* enterotoxigenic virulence genes were detected in all three studied strains.
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Key words: Meat products, toxigenic bacteria, enterotoxins, virulence genes

1. Introduction

Toxigenic bacterial species of *E. coli,* Salmonellae; coagulase positive *S. aureus* and *B. cereus* have been linked to major outbreaks of food poisoning, illness and death all over the world (**Hamed *et al*., 2015** and **Zafar *et al*., 2016)**. Bacterial toxigenesis is a major mechanism by which pathogenic bacteria produce diseases. They produce two kinds of toxins, lipopolysaccharides and protein toxins. Lipopolysaccharides are cell-associated toxins released after disruption of the cell "endotoxins", whereas protein toxins are synthesized inside the cells and then released to the target cells "exotoxins" (**Singh *et al*., 2014** and **Rudkin *et al*., 2017**).

Escherichia coli is one of the most important toxigenic bacterial pathogens in meat and its products and has been associated with numerous out breaks of disease resulting from contaminated meat products **(Datta *et al*., 2012 and Hamed *et al*., 2015).** Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. EPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia **(Russo and Johnson, 2000 and Kaper *et al*., 2004)**. It is commonly non-virulent, but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. Pathogenic *E. coli* strains have been broadly classified into two major categories; the diarrheagenic *E. coli* and the extraintestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories including Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enter aggregative *E. coli* (EAEC), diffusively adherent *E. coli* (DAEC) and Enterohaemorrhagic *E. coli* (EHEC)/Shiga toxin-

producing *E. coli* (STEC) that has the ability to produce one or more Shiga toxins **(Monaghan *et al.*, 2011).** *E. coli* heat-labile toxin LT Activation of adenylatecyclase; increasing intracellular cAMP, fluid and electrolytes secretion in intestinal epithelium leading to diarrhea (**Ma, 2016).** *E. coli* ST Toxin binding to heat-stable enterotoxins (ST) to a guanylatecyclase receptor leading to an increase in cyclic GMP (cGMP), affect electrolyte reflux **(Weiglmeier *et al.*, 2010).** In addition, numerous outbreaks of disease resulting from contaminated meat and its products with pathogenic *E. coli* have been recognized as a serious food borne pathogen that may be associated with urinary tract infections, neonatal meningitis, pneumonia, surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia **(Al- Mariri and Safi, 2014).**

Staphylococcus aureus is considered also important food borne diseases worldwide due to its ability to produce wide arrays of toxins **(Balaban and Rasooly, 2001 and Argudin *et al.*, 2010).** The most relevant characteristic of *S. aureus* is the production of heat-stable enterotoxins implicated in food-borne intoxications. Currently, 20 Staphylococcal enterotoxins (SEs) are known: 5 classical and 15 newly described **(Ono *et al.*, 2008)**. The potential cause food-borne intoxications has been reported in all classical SEs (*sea–see*) and a single new SE, *seh* **(Omoe *et al.*, 2002).**

In addition, the enterotoxigenic *Bacillus cereus* strains under certain conditions produce haemolysis, phospholipases c and enterotoxin that cause food poisoning (**Abostate *et al.*, 2006).** Food-borne diseases caused by *B. cereus* are notoriously classified as emetic and diarrheal syndromes. Furthermore, one-component toxins, such as enterotoxins T and FM, as well as cytotoxin K (*cyt*K) are thought to be involved in *B. cereus* food poisoning and are considered as the primary virulence factors in *B. cereus* diarrhea. In addition to these proteins, *B. cereus* produces sphingomyelinase, phosphatidylinositol- and phosphatidylcholine-specifc phospholipases "PIPLC and PC-PLC" (**Lund *et al.*, 2000**). Sometimes both types of symptoms are produced probably due to the synergistic effects of one or more enterotoxin (s), *B. cereus* produces emetic toxin and four other enterotoxins: hemolysin BL or *hbl*, non-hemolytic enterotoxin or *nhe* (**Lindback *et al.*, 2004).**

As food-borne bacteria specially toxigenic ones constitutes serious problems for consumers, so, the present study was conducted to estimate the prevalence of some toxigenic food-borne bacteria in meat and common meat products (beef burger, kofta; minced meat and sausage) at Kaliobia Governorate, Egypt, beside the phenotypic characterization of the isolate and determination of virulence genes in them.

2. Materials and Methods

2.1. Samples

A total of 250random samples of fresh meat and meat products *viz*: Beef burger, kofta; minced meat and sausage (50 for each), were collected from different shops at Kaliobia Governorate, Egypt.

2.2. Bacteriological examination

A total of 25 grams of each sample under examination were prepared for bacteriological examination following (**APHA, 2001**).

2.2.1. Isolation and identification of *E. coli* following (ISO16649-3, 2001):

Typical *E. coli* colonies on Tryptone Bile Glucournide (TBX) medium appeared as blue colonies, were picked up for identification morphologically by Gram stain; biochemical tests and serologically by slide agglutination test using *E. coli* antisera (table, 1) of DENKA SEIKEN CO., LTD. TOKYO, Japan according to **Edward and Ewing (1972)** and **Markey *et al.* (2013).**

Table (1): Antisera used in serological identification of *E. coli*

Polyvalent Sera	Monovalent sera						
Polyvalent 1	O1	O26	O86a	O111	O119	O127a	O128
Polyvalent 2	O44	O55	O125	O126	O146	O166	
Polyvalent 3	O18	O114	O142	O151	O157	O158	
Polyvalent 4	O6	O27	O78	O148	O159	O168	
Polyvalent 5	O20	O25	O63	O153	O167		
Polyvalent 6	O8	O15	O115	O169			
Polyvalent 7	O28ac	O112ac	O124	O136	O144		
Polyvalent 8	O29	O143	O152	O164			

H-sera: H2, H4, H6, H7, H11, H18 and H21.

2.2.2. Isolation and identification of *S. aureus* strains following FDA (2001):

Suspected *S. aureus* colonies that appeared as circular, smooth, convex, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone on Baird-Parker agar were identified morphologically by Gram stain, biochemically, and coagulase activities according to **Paul *et al.* (2009) and Markey *et al.* (2013).**

2.2.3. Isolation and identification of *B. cereus* strains following Rhodehamel and Harmon (2001):

Typical *B. cereus* colonies (blue, turquoise to peacock blue, about 5 mm in diameter and surrounded by a zone of egg yolk precipitation on Polymyxin – pyruvate-Egg yolk-Mannitol-Bromothymol blue agar "PEMBA" were picked up for identification morphologically by Gram stain and biochemical tests following **Paul *et al.* (2009)** and **Markey *et al.* (2013).**

2.2.4. Detection of Virulence genes in *E. coli; S. aureus* and *B. cereus* strains by PCR

Genotyping detection of shiga toxin1 gene (*stx*1); shiga toxin2 gene (*stx*2) and edema verotoxin gene (*vt*2*e***)** in 6 random *E. coli* strains; enterotoxin Agene (*sea*); enterotoxin B gene (*seb*); enterotoxin C gene (*sec*); enterotoxin D gene (*sed**)*** and enterotoxin Egene **(***see***)** *S. aureus* in 5 random *S. aureus* strains, beside, non-hemolytic enterotoxin gene (*nhe*); cytotoxic K gene (*cyt* K) and emetic toxin cereulide, cereulidesynthetasegene (*ces*) in 3 random *B. cereus* strains using uniplex, duplex and multiplex polymerase chain reaction, following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR mastermix (Takara, Japan) and 1. 5% agarose gel electrophoreses **(Sambrook *et al.*, 1989)** using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (2).

Table (2): Primers sequences, target genes, amplicons sizes and cycling conditions

Target M.O.	Target gene		Primer sequence (5'-3')	Amplified segment (bp.)	Primary denaturation	Amplification (35 cycles)			Final extension	References
						Secondary denaturation	Annealing	Extension		
E. coli	*stx1*	F **R**	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	**614 bp**	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Dipineto *et al.*, 2006**
	stx2	F **R**	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	vt2e	F **R**	CCAGAATGTCAGATAACTGGCGAC GCTGAGCACTTTGTAACAATGGCTG	322 bp	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Orlandi *et al.*, 2006**
S. aureus	***Sea***	F **R**	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	**102 bp**	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Mehrotra *et al.*, 2000**
	Seb	F **R**	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	Sec	F **R**	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	**451 bp**	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	Sed	F **R**	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	See	F **R**	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
B. cereus	*Nhe*	F **R**	AAG CIG CTC TTC GIA TTC ITI GTT GAA ATA AGC TGT GG	766 bp	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Ehling-Schulz *et al.*, 2006**
	cytK	F **R**	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421 bp	**94˚C 5 min.**	**94˚C 30 sec**	**49˚C 40 sec**	72˚C 45 sec.	**72˚C 10 min.**	
	Ces	F **R**	GGTGACACATTATCATATAAGGTG GTAAGCGAACCTGTCTGTAACAACA	1271 bp	**94˚C 5 min.**	**94˚C 30 sec**	**49˚C 40 sec.**	72˚C 1.2 min.	**72˚C 12min**	

3-Results

The results of bacteriological examination of examined meat and meat product samples; and genotyping detection of virulence genes in *E. coli; S. aureus* and *B. cereus* isolated strains were tabulated in Tables (3 & 4) and Figures (1-6).

Table (3): Prevalence of *E. coli*; *S. aureus* and *B. cereus* isolates in examined samples

Samples	**Fresh meat**		**Beef Burger**		**Kofta**		**Minced meat**		**Sausage**		**TOTAL**	
Isolates	**No.**	**%[1]**	**No.**	**%[1]**	**No.**	**%[1]**	**No.**	**%[1]**	**No.**	**%[1]**	**No.**	**%[2]**
E. coli	**4**	**8.0**	**3**	**6.0**	**6**	**12.0**	**7**	**14.0**	**5**	**10.0**	**25**	**10.0**
S. aureus	**8**	**16.0**	**4**	**8.0**	**12**	**24.0**	**9**	**18.0**	**8**	**16.0**	**41**	**16.4**
B. cereus	**1**	**2.0**	**3**	**6.0**	**7**	**14.0**	**4**	**8.0**	**6**	**12.0**	**21**	**8.4**
TOTAL	**13**	**26.0**	**10**	**20.0**	**25**	**50.0**	**20**	**40.0**	**19**	**38.0**	**87**	**34.8**

%[1] Percentage in relation to total number of each sample (50)
%[2]Percentage in relation to total number of samples (250)

Table (4): Serological typing of *E. coli* strains isolated from different examined samples

Samples	**Fresh meat**		**Beef Burger**		**Kofta**		**Minced meat**		**Sausage**		**TOTAL**	
***E.coli* serotype**	**NO.**	**%[1]**	**NO.**	**%[1]**	**NO.**	**%[1]**	**NO.**	**%[1]**	**NO.**	**%[1]**	**NO.**	**%[1]**
O_{55}:H_7	1	4.0	1	4.0	2	8.0	2	8.0	1	4.0	7	28.0
O_{111}:H_4	1	4.0	0	0.0	1	4.0	1	4.0	0	0.0	3	12.0
O_{125}:H_{18}	1	4.0	0	0.0	1	4.0	2	8.0	1	4.0	5	20.0
O_{126}:H_7	0	0.0	0	0.0	1	4.0	1	4.0	1	4.0	3	12.0
O_{128}:H_{27}	1	4.0	1	4.0	0	0.0	0	0.0	0	0.0	2	8.0
O_{142}:H_2	0	0.0	1	4.0	0	0.0	0	0.0	1	4.0	2	8.0
O_{158}:H_2	0	0.0	0	0.0	1	4.0	1	4.0	1	4.0	3	12.0
TOTAL	4	16.0	3	12.0	6	24.0	7	28.0	5	20.0	25	100.0

%[1] Percentage in relation to total number of examined *E. coli* (25)

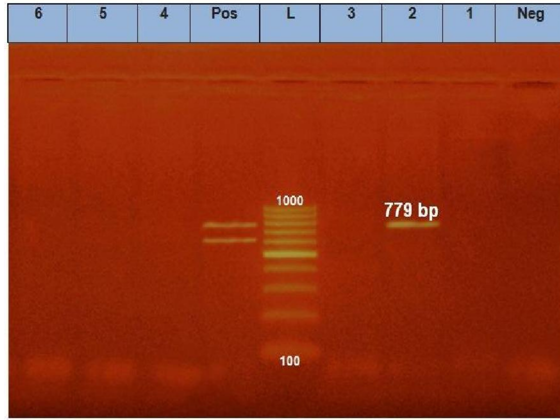

Fig. (1): Agarose Gel electrophoresis of shiga toxin 1 and shiga toxin 2 genes (*stx*1 and *stx*2) of *E. coli*
Lane L: 100-1000 bp. DNA Ladder
Neg.: Negative control (*S. aureus* ATCC25923), Pos.: Positive control (*E.coli* AJ413986)
A. *stx*1**:**
Lane 1 -6: *E. coli* (Negative for***stx*1** gene at 614 bp.)
B. *stx*2
Lane 1, 3 -6: *E. coli* (Negative) Lane 2: *E. coli* (Positive for *stx*2at 779bp.)

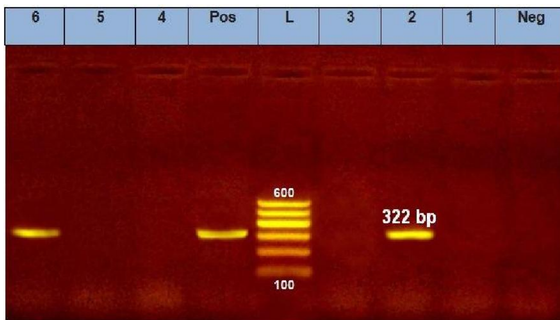

Fig. (2): Agarose Gel electrophoresis of edema verotoxin gene (*Vt2e*) of *E. coli*
Lane L: 100-600 bp. DNA Ladder
Neg.: Negative control (*S. aureus* ATCC25923)
Pos.: Positive control (*E.coli* AJ413986)
Lane 1, 3 -5: *E. coli* (Negative)
Lane2, 6: *E. coli* (Positive for *Vt2e* at 322 bp.)

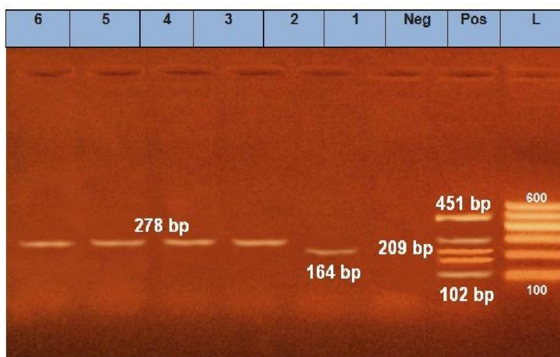

Fig. (3): Agarose Gel electrophoresis of Enterotoxin (*sea, seb, sec, sed, see*) genes of *s.aureus*
Lane L: 100-600 bp. DNA Ladder Neg.: Negative control (*E.coli* AJ413986) **Pos.: Positive control (***S. aureus* ATCC25923)
A. *sea***:**
Lane 1-5: ***S. aureus*** (Negative **for *sea* at 102 bp**.)
B. *seb***:**
Lane 1: *S. aureus* (Positive for *seb* at 164 bp.) Lane 2-5: *S. aureus* (Negative)
C. *sec*:
Lane 1-5: ***S. aureus*** (Negative **for *sec* at 451 bp**)
D. *sed***:**
Lane 1: *S. aureus* (Negative). **Lane2-5: *S. aureus* (Positive for *sed* at 278 bp.)**
E. *see***:**
Lane 1-5: ***S. aureus*** (Negative **for *see* at 209 bp.**)

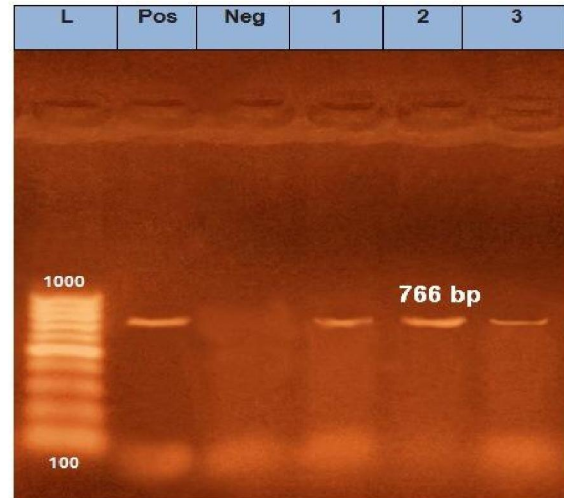

Fig. (4): Agarose Gel electrophoresis of non-hemolytic enterotoxin (*nhe*) gene
Lane L: 100-1000 bp. DNA Ladder
Neg.: Negative control (*E. coli* AJ413986) Pos.: Positive control (*B.cereus* form Ahri.)
Lane 1 -3: *B.cereus* (Positive at 766 bp.)

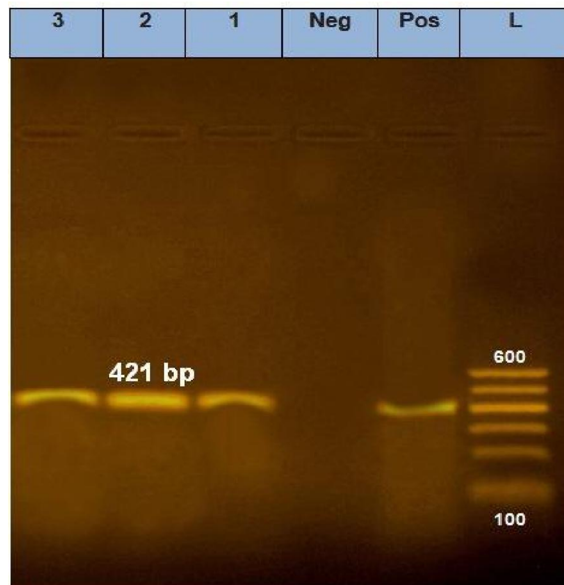

Fig. (5): Agarose Gel electrophoresis of cytotoxic K (*cytK*) gene
Lane L: 100-600 bp. DNA Ladder
Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri.)
Lane 1 -3: *B.cereus* (Positive at 421 bp.)

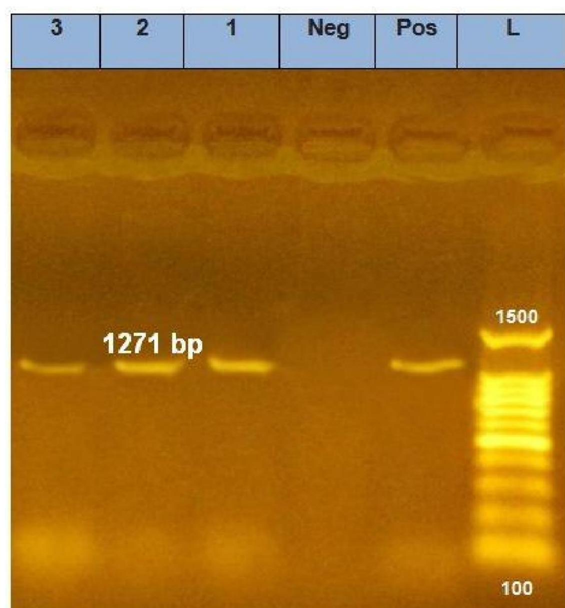

Fig. (6): Agarose Gel electrophoresis of cereulidesynthetase gene (*ces*)
Lane L: 100-1500 bp. DNA Ladder
Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri.)
Lane 1 -3: *B.cereus* (Positive at 1271 bp.)

4- Discussion

Pathogenic, mainly toxigenic bacterial species of *E. coli*; coagulase positive *S. aureus* and *B. cereus* have been linked to major outbreaks of food poisoning, illness and death all over the world (**Son *et al.*, 2014** and **Zafar *et al.*, 2016**).

The results of bacteriological examination of examined samples (Table, 3) revealed that, a total of 87 (34.8%) isolates of *S. aureus* isolates (41/16.4%); *E. coli* (25 /10.0%) and *B. cereus* (21/8.4%) were recovered from 250 examined samples. Nearly similar results were recorded by **Abd El-Tawab *et al.* (2015a & b); Binsy *et al.* (2016)**; **El-Rais, Eman (2018)** and **El-Sayed (2019)**.

Pathogenic strains of *E. coli* affecting humans are responsible for intestinal diseases (gastroenteritis) and extra intestinal infections, which include urinary tract infections (UTI), bacteremia, and neonatal meningitis *E. coli* accounts for more than 90% of all uncomplicated UTIs (**Binsy *et al.*,2016**). Twenty five *E. coli* strains were isolated from minced meat samples (7/14%) followed by kofta (6/12.0%); sausage (5/10.0%); fresh meat (4/8.0%) and beef burger samples (3/6.0%). Nearly similar results were obtained by **Tarabees *et al.* (2015); Armany *et al.* (2016); El-Rais, Eman (2018)**; **El-Sayed (2019)** and **El-Shora, Heba (2019)**. Meanwhile, these results were disagreed with those of **Gwida *et al.* (2014); Abd El-Tawab *et al.* (2015b); Adwan *et al.* (2015)** and **Abd El Salam, Marwa (2019)** who isolated *E. coli* from raw meat and meat products with high incidence. In addition, the results were disagreed with **Siriken *et al.* (2006)** and **Hamed *et al.* (2015)** who failed to isolate *E. coli* from beef burger and sausage samples. The serological examination of 25 isolated *E. coli* isolates (Table, 4) cleared that, seven isolates were typed as O55: H7 (two from each samples of kofta, minced meat and one from each samples fresh meat, beef burger, sausage); three O_{111}: H_4 (one from each samples of fresh meat, kofta, and minced meat); five O_{125}: H_{18}(two from minced meat; one from each samples of fresh meat; kofta and sausage); three O126: H7(one from each samples of kofta; minced meat and sausage); two O128: H27 (one from each samples of fresh meat and beef burger); two O142: H2(one from each samples of beef burger and sausage) beside three O158: H2 (one from each samples of kofta; minced meat and sausage samples). These results came in harmony with those of **Abd El-Tawab *et al.* (2015b)**; **Tarabees *et al.* (2015); El-Rais, Eman (2018)** and **El-Sayed (2019)** who detected the same serotypes of *E. coli* from meat and meat product samples. The recovery of *E. coli* from meat and its products samples indicates fecal contamination and implies that other pathogens of fecal origin may be present. The increased incidence of *E. coli* in the examined samples may be due to mishandling during production, processing and distribution or to the use of contaminated water during evisceration and slaughtering **(Gwida *et al.*, 2014).**

A total of 41 *S. aureus* isolates were mostly isolated from kofta samples (12 / 24.0%) followed by minced meat (9/ 18.0%), sausage, fresh meat (8/16.0% for each) and beef burger samples (4/8.0%). These results came in accordance with those obtained by **Goja *et al.* (2013); Abd El-Tawab *et al.* (2015); Armany *et al.* (2016); El-Rais, Eman (2018)** and **El-Shora, Heba (2019)**. Meanwhile, these results disagreed with those of **Abd El -Hady (2015); Adwan *et al.* (2015) and Tarabees *et al.* (2015**) who isolated *S. aureus* from fresh meat and meat products with high incidence. Also, disagreed with those recorded by **Wehab and Hegazy (2007)** and **Kalantari *et al.* (2012)** who failed to isolate *S. aureus* from beef burger and beef sausage samples. The presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils and they grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhoea and gastroenteritis among consumers (**Plaatjies *et al.*, 2004).**

Bacillus cereus is one of the potential spoilage bacteria associated with meat products and the presence of them with high levels indicates a potential risk of producing toxins. The results of *B. cereus*

isolation cleared that, 21 strains were isolated mostly from kofta (7/14.0%) followed bysaus age (6/12.0%); minced meat (4/8.0%); beef burger (3/6.0%) and fresh meat samples (1/2.0%). Nearly similar results were obtained by **Rather *et al.* (2011)**; **Tewari *et al.* (2015)** and **Ibrahim, Hemmat *et al.* (2014b)**. But disagree with those obtained by **Samir *et al.* (2012)**; **Abd El-Tawab *et al.* (2015a); Mohamed and Ghanyem (2015); Salim, Dalia *et al.* (2015); Soleimani *et al.* (2017)**) and **El-Shora, Heba (2019)** who isolated *B. cereus* from fresh meat and meat products with high incidence.

The PCR technique is capable of identifying the pathogenic *E. coli*; *S. aureus* and *B. cereus* isolates. Based on the fact that virulence genes varies not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence genes of isolated *E. coli*; *S. aureus* and *B. cereus* strains (**Seong *et al.*, 2008; Byrne *et al.*, 2014; Savic *et al.*, 2015** and **Saleh *et al.* (2016)).** So, the present study was directed mainly to recognize some virulence genes that may play a role in toxigenicity of these isolates by using one of the recent developments molecular biological techniques (PCR). These genes were shiga toxin1 gene (*stx*1); shiga toxin2 gene (*stx*2) and edema verotoxin gene (*vt*2*e*) for *E. coli*; enterotoxin Agene (*sea*); enterotoxin B gene (*seb*); enterotoxin C gene (*sec*); enterotoxin D gene (*sed*) and enterotoxin Egene (*see*) for *S. aureus* and non-hemolytic enterotoxin gene (*nhe*); cytotoxic K gene (*cyt*K) and emetic toxin cereulide, cereulidesynthetase gene (*ces*) for *B. cereus* strains.

Regarding to *E. coli* isolates, the strains producing *stx*1, *stx*2, *stx*2c and *stx*2d are associated with haemorrhagic enteritis in humans. The toxin *stx*2 is produced in the intestine but isabsorbed and carried via the bloodstream to the target cells, usually endothelial cells of the small arteries (**Markey *et al.*, 2013**). The results of PCR amplification of shiga toxin1 gene (*stx*1); shiga toxin2 gene (*stx*2) in *E. coli*isolates (Fig**.**, 1) showed that, the *stx*1 gene was failed to be amplified in all studied isolates at 614 bp., meanwhile, *stx*2 gene was amplified in one *E. coli*isolates giving product of 779 bp. Similar findings were recorded by **Byrne *et al.* (2014)**; **Mohammed and El Dahshan (2016)** and **Abd El-Badiea, Zeinab *et al.* (2019)**. But disagree with those obtained by **Abdel-Rhman *et al.*, (2015)** and **El-Shora, Heba (2019)** who failed to detect*stx*1and *stx*2 in examined *E. coli* isolates. *E. coli* strains producing shiga-toxin 2e (*vt2e* also known as *stx*2e) play an important role in the pathogenesis of edema disease. One target of the *vt2e* toxin is endothelial cells of small blood vessels resulting in edema. The results of PCR amplification of edema verotoxin (*vt2e*) gene in *E. coli*isolates (Fig**.**,2) cleared that, *vt2e* gene was amplified in two *E. coli* strains giving product of 322 bp. Similar findings were obtained by **Blanco *et al.* (2006)** and **Gyles (2014)**.

Meanwhile, for *S. aureus* isolates; the results of PCR amplification of enterotoxin genes (*sea*; *seb*; *sec*; *sed* and *see*) in *S. aureu s* isolates (Fig**.**,3) cleared that, the *seb* gene was amplified in one *S. aureus* strain giving product of 164 bp. and the *sed* gene was amplified in four *S. aureus* strains giving product of 278 bp. But, the *sea*; *sec* and *see* genes were failed to be amplified in all studied strains at102 bp., 451 bp. and 209 bp., respectively. These results for *seb* and *sed* genes came in harmony with **Kitai *et al.* (2005)**; **Abdalrahman *et al.* (2015); Saleh *et al.* (2016)** and **Moustafa *et al.* (2017)**. Meanwhile, the results for other genes (*sea*; *seb*; *sec*; *sed* and *see*) came in accordance with **Fe Bler *et al.* (2011)** and **El-Shora, Heba (2019)** who failed to detect all studied genes in *S. aureus* strains. The mechanisms of SEs causing food poisoning are not clearly known. However, it is believed that SEs directly affect intestinal epithelium and vagus nerve causing stimulation of the emetic center **(Hennekinne *et al.*, 2012).**

In addition, for *B. cereus* isolates; the result of PCR amplification of then on-hemolytic enterotoxin (*nhe*) gene in *B. cereus* isolates (Fig., 4) appeared that, the *nhe* gene was amplified in all three studied *B. cereus* strains giving product of 766 bp. These results were agreed with those obtained by **Forghani *et al.* (2014); Tewari *et al.* (2015)**; **Rather *et al.* (2016)** and **El-Shora, Heba (2019)**. The result of PCR amplification of the cytotoxic K (*cyt* K) genein *B. cereus* isolates (Fig., 5) showed that, the *cyt* Kgene was amplified in all three studied *B. cereus* strains giving product of 421 bp. The results came in harmony with those of **Ngamwongsatit *et al.* (2008); Tewari *et al.* (2015)**; **Rather *et al.* (2016)** and **Abd El-Wahaab, Shimaa (2018)** who detected *cyt* Kgene from Enterotoxigenic strains of *B. cereus* isolated from meat and meat products**.** The result of PCR amplification of the cereulidesynthetase (*ces*) in *B. cereus* isolates (Fig., 6) cleared that, the *ces* gene was amplified in all three studied *B. cereus* strains giving product of 1271 bp. Similar findings were recorded by **Seong *et al.* (2008); Kim *et al.* (2010); Lim *et al.* (2011); Chon *et al.* (2012); Lee *et al.* (2012); Salim-Dalia *et al.* (2015); Savic *et al.* (2015); Aubaidand Dakel (2017) and El-Sayed (2019)**. Meanwhile, **Aragon *et al.* (2008); Ankolekar *et al.* (2009); Ahaotu *et al.* (2013)** and **El-Shora, Heba (2019)** failed to detect ***ces* ge**ne in *B. cereus* strains. The diarrheal and emetic syndrome due to *B. cereus* food poisoning, manifested via the release of one or more enterotoxins; non-hemolytic enterotoxin (*nhe*),

cytotoxin K (*cyt*K) and cereulide (*ces*) heat-stable emetic toxin **(Moravek *et al.*, 2006).**

Finally, each *E. coli*; *S. aureus* and *B. cereus* isolates harbored at least one of the enterotoxin genes indicating their pathogenic nature, which must be considered as serious healthhazard and I is high probability of the potential transmission of enterotoxigenic studied strains to humans from the food chain, more particularly through contamination of meat and meat products. So, PCR is a rapid and highly sensitive diagnostic method for detection of *E. coli*; *S. aureus* and *B. cereus* virulence genes and to differentiate between enterotoxigenic and non-enterotoxigenic isolates, therefore, PCR amplification using specific primers would facilitate direct detection of these isolates in meat and its products. Moreover, the recorded results showed a high bacterial load beside a relatively high rate of pathogens, this may be due to mishandling and the negligence of hygienic aspects. So, it was concluded that, *E. coli*; *S. aureus* and *B. cereus* strains are enterotoxigenic ones and they are meat-borne pathogens of public health importance.

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