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DETECTION OF SOME OF VIRULENCE GENES IN *SALMONELLA KENTUCKY* ISOLATED FROM POULTRY

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ABSTRACT

Salmonella Kentucky has an increasing world wide spread among human and animals which causes a great problem. Twenty six *Salmonella Kentucky* strains isolated from different samples during 2011 - 2016, twenty two from chicken and four from quail. In the present work we study the genetic diversity through screening of 11 virulence genes genes (*inv*A, *avr*A, *ssa*Q, *mgt*C, *sii*D, *sop*B, *gip*A, *sod*C1, *sop*E1, *spv*C, and *bcf*C) by PCR. The *inv*A were detected in 100% of the *Salmonella* strains; but 92.3% of strains carry *sop*B, 88.4% of strains were carry *avr*A, *bcf*C and *ssa*Q, *mgt*C (80.7%) , *sop*E and *sod*C (19.2%), *sii*D (11.5%), *spv*C (3.8%), while no one carry *gip*A. These results show the presence of virulence genes in *Salmonella Kentucky* with potential poultry and public health hazard.

Key words: *Salmonella*, *Salmonella Kentucky*, *avr*A, *ssa*Q, virulence genes.

INTRODUCTION

Salmonella is a major zoonotic pathogen in Europe, causing approximately 152,000 confirmed human infections in 2007 (Anonymous, 2009).

Salmonellosis is world wide spread among human and animals caused by different serovars belong to *Salmonella* enterica subspp. Enterica as Enteritidis, Typhimurium, Newport, and Javiana, *Salmonella Kentucky* ST198 has an increasing multiple drug resistant which consequently showing it has a public health hazard (LeHello *et al*., 2011).

Food and Safety Inspection Service (FSIS), from 2000 to 2009 reported that *Salmonella* Kentucky, Enteritidis, Heidelberg and Typhimurium are commonly found in broilers and ground chicken (Andino and Hanning, 2015).

Most virulence genes of *Salmonella* are clustered in regions distributed over the chromosome called *Salmonella* pathogenicity islands (SPI) (van Asten and van Dijk, 2005).

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These gene-clusters might be acquired by *Salmonella* from other species through horizontal gene transfer, this hypothesis is based on the significant difference in GC content of the islands compared to that of the residual genome and the remnants of bacteriophages or transposon insertion sequences that often mark the borders of the islands (van Asten and van Dijk, 2005).

Amplification of *inv*A gene now has been recognized as an international standard for detection of *Salmonella* genus (Malorny *et al.,* 2003).Invasion gene (*inv*A) responsible for intestinal mucosa invasion by all Salmonellae (Fluit, 2005; Chuanchuen *et al*., 2010), this gene which is chromosomally located aids attachment of the pathogen to the epithelial cells (Galán and Curtiss, 1989).

The Sop proteins (*sop*A-E) (sop) and the heat-labile *Salmonella* enterotoxin (stn) are effector proteins that is integrated in pathogenesis of *Salmonella* through survival and replication (van Asten and van Dijk, 2005).

There are several virulence factors contributing to *Salmonella* adhesion and invasion mechanism, as *Salmonella* plasmid virulence (*spv*) operon, which consists of five genes (spvRABCD), potentiates the systemic spread of the pathogen and aids in its replication in extra-intestinal sites (Zou *et al*., 2012). spvR is a positive regulatory protein essential for the expression of the other spv genes (Guiney *et al*.,

1995) while *spv*B product ADP-ribosylates actin, make imbalance of the eukaryotic cell (Lesnick *et al*., 2001). Experiments have shown that *spv*B together with *spv*C is responsible for virulence to *Salmonella* Typhimurium when administered subcutaneously to mice (Matsui *et al*., 2001).

*mgt*C gene encodes *Mgt*B Mg2+ transporter and helps *Salmonella* to survive within macrophages (Alix and Blanc-Potard, 2007).

The *sig*DE operon encodes *Sig*D (*Sop*B), a multifaceted effector that is involved in many steps of pathogenesis (Fàbrega and Jordi, 2013).

While the fimbrial gene *bcf*C has a role in cell invasion (Huehn *et al.*, 2010). Induction of cell apoptosis to limit the host's inflammatory responses is mediated by the *avr*A gene (Borges *et al*., 2013).

Aim of this work is to determine the extent to which virulence genes (*inv*A, *avr*A, *ssa*Q, *mgt*C, *sii*D, *sop*B, *gip*A, *sod*C1, *spv*C, *sop*E1, *bcf*C) existence in *Salmonella* Kentucky from avian origin that may pose a risk to the human population and poultry in Egypt.

MATERIALS AND METHODS

Bacterial isolates:
Collection of *Salmonella* Kentucky strains from July 2011 to May 2016 was done during routine examination of different samples submitted to reference laboratory for veterinary quality control on poultry production, a total of 26 *Salmonella* Kentucky strains were collected from internal organs, feed, embryonated eggs, drag swabs and paper lining chick box, twenty two from chicken and four from quail.

Bacterial isolation and identification:
The detection and identification of *Salmonella* isolates was done according to ISO 6579/cor.1.2004 and by serotyping of all the *Salmonella* isolates were done by slide agglutination using commercial O and H antisera (Difco Laboratories, Detroit, MI, USA) in accordance with the Kauffmann–White typing scheme and ISO/TR 6579-3:2014**.**

Polymerase chain reaction (PCR):
DNA extraction was performed using QIAamp DNA mini kit (Qiagen, Germany, GmbH Catalogue no.51304).

Oligonucleotide primer: primers were used supplied from metabion (Germany) and PCR conditions was mentioned as in Table (1).

All samples were confirmed by using Conventional PCR technique by using *inv*A gene.

Virulence gene detection:
Conventional PCR technique was used for detection of virulence determinants by detection of 10 virulence genes (*avr*A, *ssa*Q, *mgt*C, *sii*D, *sop*B, *gip*A, *sod*C1, *sop*E1, *spv*C, and *bcf*C) in the 26 *Salmonella* Kentucky strains by conventional PCR technique.

Isolates were purified on LB (luria-Bertani) agar and subsequently grown overnight at 37°C in LB broth.

PCR amplification: a volume of 25 µL PCR reaction containing 12.5 µL of Emerald Amp Max PCR Master Mix (Emerald, Japan), 1 µL of each primer of 20 pmol concentrations, 4.5 µL of Depic water and 6 µL of template was used in a Biometra thermal cycler. The reference strains provided by the External Quality Assurance Services (EQAS) were used as positive controls of S. Kentucky. DNA of the negative control (*E. coli* NCIMB 50034).

Analysis of the PCR products:
The PCR products were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V\cm. for gel analysis 5 µL of the products was loaded in each gel slot. Gene ruler 100-1000 bp ladder. Thermo scientific was used to determine the fragment size. The gel was photographed using a gel documentation system (applied).

Table 1: Virulence factor targets and primers, including nucleotide sequences, PCR conditions, and references.

Gene designation	Location on SP1/gene function	Oligonucleotide sequences (5\`–3\`)	PCR conditions [a]			Product Size (bp)	References
			Denaturing	Annealing	Extension		
*inv*A	Type III secretion system apparatus SPI-1/invasion of macrophages	**F:** GTG AAA TTA TCG CCA CGT TCG GGCAA	94°C for 60 seconds	64ºC for 30 seconds	72ºC for 30 seconds[b]	284	**Salehi *et al*., `2005**
		R:TCA TCG CAC CGT CAA AGG AACG					
*avr*A	SPI-1/controls *Salmonella*-induced inflammation	**F:**CCT GTA TTG TTG AGC GTC TGG	95ºC for 30 seconds	58ºC for 30 seconds	72ºC for 30 seconds[b]	422	**Huehn *et al*., 2010**
		R:AGA AGA GCT TCG TTG AAT GTCC					
*ssa*Q	SPI-2/secretion system apparatus protein, component of second T3SS	**F:**GAA TAG CGA ATG AAG AGC GTCGTC C	"	"	"	455	**Huehn *et al*., 2010**
		R:CAT CGT GTT ATC CTC TGT CAG C					
*mgt*C	SPI-4/Mg^{2+} uptake	**F**:TGA CTA TCA ATG CTC CAG TGA AT	"	"	"	677	**Huehn *et al*., 2010**
		R:ATT TAC TGG CCG CTA TGC TGT TG					
*sii*D (*Spi4*D)	Type I secretion/SPI-4	**F**:GAA TAG AAG ACA AAG CGA TCA TC	"	"	"	655	**Hauser *et al*., 2011**
		R:GCT TTG TTC ACG CCT TTC ATC					
*sop*B	SPI-5/inositol polyphosphate, phosphatase that promotes macropinocytosis, regulates SCV localization, and promotes fluid secretion	**F**: TCA GAA GRC GTC TAA CCA CTC	"	"	"	517	**Huehn *et al*., 2010**
		R:TAC CGT CCT CAT GCA CAC TC					
*gip*A	Gifsy-1 bacteriophage/Peyer's patch-specific virulence factor	**F:**ACG ACT GAG CAG CGT GAG	"	"	"	518	**Huehn *et al*., 2010**
		R:TTG GAA ATG GTG ACG GTA GAC					
*sod*C1	Gifsy-2 bacteriophage/periplasmic Cu, Zn-superoxide dismutases	**F:**CGG GCA GTG TTG ACA AAT AAAG	"	"	"	424	**Huehn *et al*., 2010**
		R:TGT TGG AAT TGT GGA GTC					
*sop*E1	Cryptic bacteriophage/promotes membrane ruffling and disrupts tight junctions	**F:**ACT CCT TGC ACA ACC AAA TGC GGA	"	"	"	422	**Huehn *et al*., 2010**
		R:TGT CTT CTG CAT TTC GCC ACC					
*spv*C	pSLT/A phosphothreonine lyase required for complete virulence in murine models	**F:**ACC AGA GAC ATT GCC TTC C	"	"	"	467	**Huehn *et al*., 2010**
		R:TTC TGA TCG CCG CTA TTC G					
*bcf*C	Chromosome/bovine colonization factor, fimbrial usher	**F:**ACC AGA GAC ATT GCC TTC C	95ºC for 30 seconds	53ºC for 30 seconds	72ºC for 30 seconds[b]	467	**Huehn *et al*., 2010**
		R:TTC TGC TCG CCG CTA TTC G					

[a] PCR was done for 35 cycles.

[b] After 30 cycles, final extension step of 4 minutes at 72ºC was performed.

[c] SCV, Salmonella-containing vacuole.

RESULTS

1. Bacterial isolates:

A total of 26 *Salmonella* Kentucky strains; twenty two from chicken (2 drag swabs, 1 table eggs, 1 embryonated eggs, 6 internal organs, 2 feed, 10 paper lining chick box) and four from cloacal swabs from quail.

2. Virulotyping:

All isolates were screened using PCR analysis for the presence or absence of 11 selected virulence genes (Table 1). The *inv*A were detected in 100% of the *Salmonella* strains; SopB 92.3%. 88.4% of strains were carry, *avr*A, *bcf*C and *ssa*Q, *mgt*C (80.7%), *sop*E and *sod*C (19.2%), *sii*D (11.5%), *spv*C (3.8%), while no one carry *gip*A.

Table 2: Distribution of the virulence genes among *Salmonella* Kentucky strains.

No.	source	invA	*mgtC*	*sopB*	*avrA*	*bcfC*	*spvC*	*sopE1*	*gip*A	*sii*D	*ssaQ*	*Sod*C1
1	chicken	+	+	+	+	+	-	-	-	-	+	-
2	chicken	+	+	+	+	+	-	-	-	-	+	-
3	chicken	+	+	+	+	+	-	-	-	-	+	+
4	chicken	+	+	+	+	-	-	-	-	-	+	-
5	chicken	+	-	+	+	-	-	-	-	-	-	-
6	chicken	+	+	+	+	+	-	-	-	-	+	-
7	chicken	+	+	+	+	+	-	-	-	-	+	-
8	chicken	+	+	+	+	+	-	-	-	-	+	-
9	chicken	+	+	+	+	+	-	-	-	-	+	+
10	chicken	+	+	+	+	+	+	-	-	-	+	+
11	chicken	+	+	+	+	+	-	-	-	-	+	+
12	chicken	+	+	+	-	+	-	-	-	-	+	-
13	chicken	+	+	+	+	+	-	-	-	-	+	-
14	chicken	+	+	+	+	+	-	-	-	-	+	-
15	chicken	+	+	+	+	+	-	-	-	-	-	-
16	chicken	+	-	+	+	+	-	-	-	-	-	-
17	chicken	+	+	+	+	+	-	+	-	+	+	-
18	chicken	+	+	+	+	+	-	+	-	+	+	-
19	chicken	+	+	+	+	+	-	+	-	-	+	-
20	chicken	+	+	+	+	+	-	+	-	-	+	-
21	chicken	+	-	+	+	+	-	-	-	-	+	-
22	chicken	+	+	+	+	+	-	+	-	+	+	-
23	Quail	+	-	-	-	-	-	-	-	-	+	-
24	Quail	+	-	-	-	+	-	-	-	-	+	-
25	Quail	+	+	+	+	+	-	-	-	-	+	-
26	Quail	+	+	+	+	+	-	-	-	-	+	+
Total		**26**	**21**	**24**	**23**	**23**	**1**	**5**	**0**	**3**	**23**	**5**
%		**100**	**80.8**	**92.3**	**88.5**	**88.5**	**3.8**	**19**	**0**	**11.5**	**88.5**	**19**

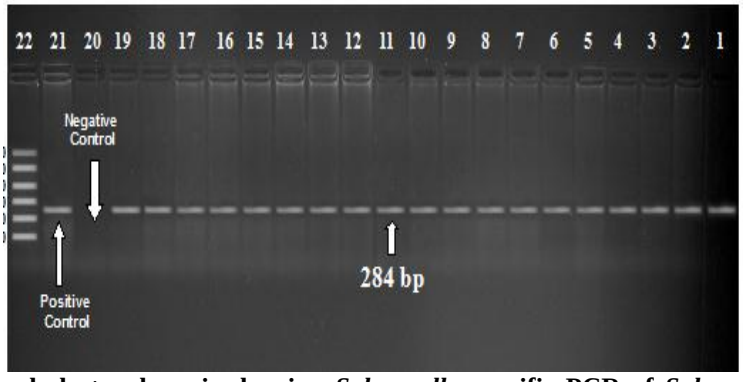

Photo (1). Agarose gel electrophoresis showing *Salmonella* specific PCR of *Salmonella* Kentucky using primer set for the *invA* (284 bp) gene

Lanes 1 - 19: positive samples of *Salmonella* Kentucky
Lane 20: Negative control (*E. coli* NCIMB 50034)
Lane 21: Positive control (*Salmonella* Kentucky EQAS)
Lane 22: DNA ladder.

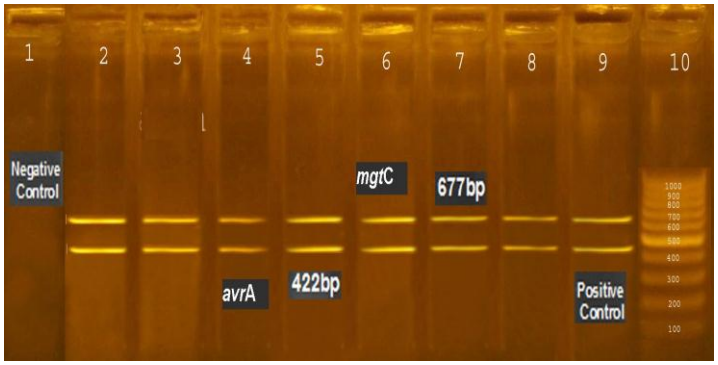

Photo (2): Agarose gel electrophoresis showing Duplex PCR with amplification of 422 bp and 677 bp bp fragments for *avrA* and *mgtC* genes of *Salmonella* Kentucky performed with their specific primers
Lane 1: DNA ladder
Lane 2: Negative control (*E. coli* NCIMB 50034)
Lane 3: Positive control (*Salmonella* Kentucky EQAS)
Lanes 4-10: positive *Salmonella* Kentucky samples for *avrA* and *mgt*C genes

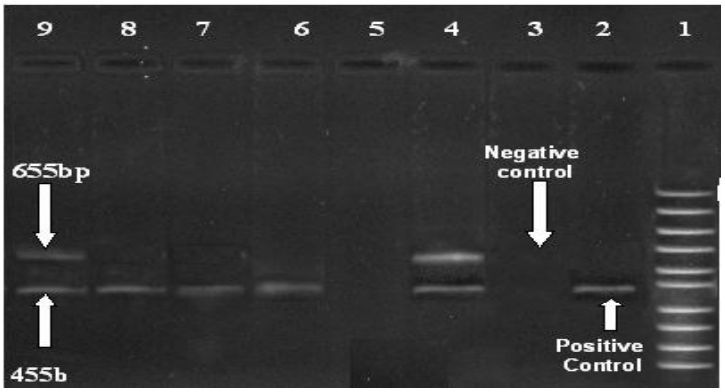

Photo (3): Agarose gel electrophoresis showing Duplex PCR with amplification of 455bp and 655bp fragments for *ssaQ* and *siiD* genes of *Salmonella* Kentucky performed with their specific primer
Lanes 1: DNA ladder
Lane 2: Positive control (*Salmonella* Kentucky EQAS)
Lane 3: Negative control (*E. coli* NCIMB 50034)
Lanes 4,9: positive *Salmonella* Kentucky samples for *ssaQ* and *siiD* genes
Lanes 6,7,8: positive *Salmonella* Kentucky samples for *ssaQ* gene
Lane 5: Negative *Salmonella* Kentucky sample for *ssaQ* and *siiD* genes

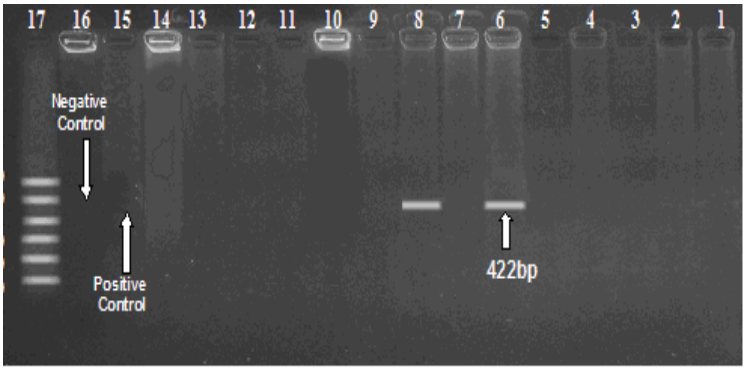

Photo (4): Agarose gel electrophoresis showing PCR amplification of the 422 bp *SopE1* gene of *Salmonella* Kentucky.
Lanes 1-5, 7, 9-14: Negative *Salmonella* Kentucky samples for *SopE1* gene
Lanes 6, 8: positive *Salmonella* Kentucky samples for *SopE1* gene
Lane 15: Positive control (*Salmonella* Kentucky EQAS)
Lane 16: Negative control (*E. coli* NCIMB 50034)
lane17: DNA ladder

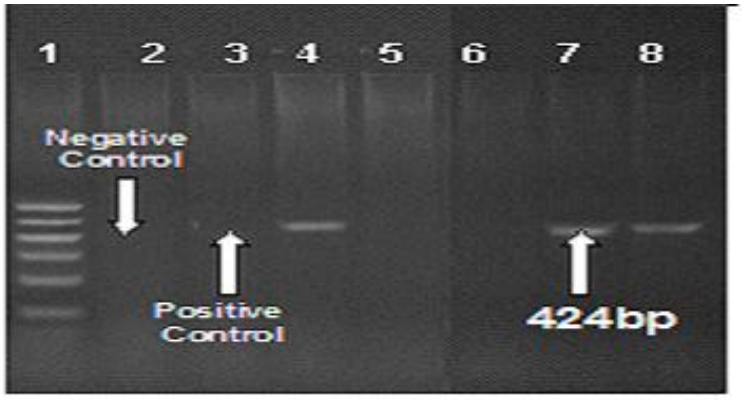

Photo (5): Agarose gel electrophoresis showing PCR with amplification of 424 bp gene *SodC1* of *Salmonella*.
Lane 1: DNA ladder
Lane 2: Negative control (*E. coli* NCIMB 50034)
Lane 3: Positive control (*Salmonella* Kentucky EQAS)
Lanes 4, 7, 8: positive *Salmonella* Kentucky samples for *SodC1* gene
Lane 5, 6: Negative *Salmonella* Kentucky sample for *SodC1* gene

Photo (6): Agarose gel electrophoresis showing PCR with amplification of 467bp fragments for *spvC* gene of *Salmonella* Kentucky performed with the specific primer
Lanes 1-10: Negative *Salmonella* Kentucky samples for *spvC* gene
Lane 11: positive *Salmonella* Kentucky samples for *spvC* gene
Lane 12: Negative control (*E. coli* NCIMB 50034)
Lane 13: Positive control (*Salmonella* Kentucky EQAS)
Lane 14: DNA ladder

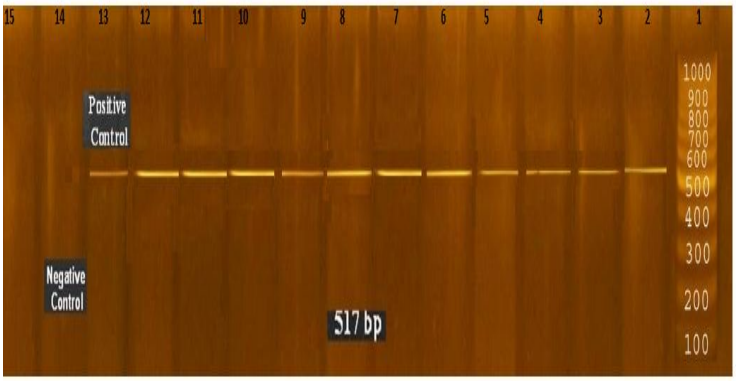

Photo (7): Agarose gel electrophoresis showing amplification product of 517 bp fragments of *sopB* gene of *Salmonella* Kentucky performed with the specific primer
Lanes 1-12: positive *Salmonella* Kentucky samples for *sopB* gene
Lane 13: Positive control (*Salmonella* Kentucky EQAS)
Lane 14: Negative control (*E. coli* NCIMB 50034)
Lane 15: DNA ladder

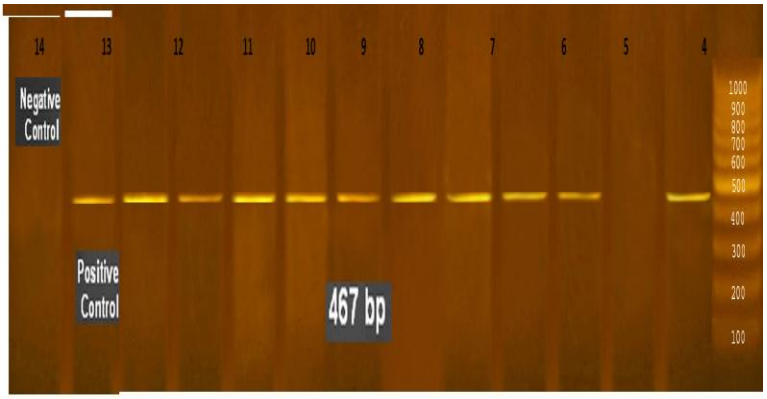

Photo (8): Agarose gel electrophoresis showing amplification product of 467 bp fragments of *bcfC* gene of *Salmonella* Kentucky performed with their primer.
Lanes 1: DNA ladder
Lanes 2,4-12: positive *Salmonella* Kentucky samples for *bcfC* gene
Lane 3: Negative *Salmonella* Kentucky sample for *bcfC* gene
Lane 13: Positive control (*Salmonella* Kentucky EQAS)
Lane 14: Negative control (*E. coli* NCIMB 50034)

Table 3: Distribution of virulence genes combinations in *Salmonella* Kentucky.

No.	source	Virulence gene combinations
1	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
2	chicken	*mgtC sopB avrA bcfC ssaQ inv*A
3	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ* Sodc1
4	chicken	*inv*A *mgtC sopB avrA ssaQ*
5	chicken	*inv*A *sopB sopB avrA*
6	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
7	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
8	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
9	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ* Sodc1
10	chicken	*mgtC sopB avrA bcfC spvC ssaQ* Sodc1 *inv*A
11	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ* Sodc1
12	chicken	*inv*A *mgtC sopB bcfC ssaQ*
13	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
14	chicken	*inv*A *mgtC sopB avrA bcfC ssaQ*
15	chicken	*inv*A *mgtC sopB avrA bcfC*
16	chicken	*inv*A *sopB sopB avrA bcfC*
17	chicken	*inv*A *mgtC sopB avrA bcfC sopE1 siiD ssaQ*
18	chicken	*inv*A *mgtC sopB avrA bcfC sopE1 siiD ssaQ*
19	chicken	*inv*A *mgtC sopB avrA bcfC sopE1 ssaQ*
20	chicken	*inv*A *mgtC sopB avrA bcfC sopE1 ssaQ*
21	chicken	*inv*A *sopB avrA bcfC ssaQ*
22	chicken	*inv*A *sopB avrA bcfC sopE1 siiD ssaQ*
23	Quail	*inv*A *ssaQ*
24	Quail	*inv*A *bcfC ssaQ*
25	Quail	*inv*A *mgtC sopB avrA bcfC ssaQ*
26	Quail	*inv*A *mgtC sopB avrA bcfC ssaQ* Sodc1

DISCUSSION

Both the presence and the dissemination *of Salmonella* spp. in foods represent an important issue to the poultry industry, since they could determine a decrease in the consumption of poultry meat, posing a threat to the national and international poultry trading (Ikuno *et al*., 2004).

S. Kentucky is widely distributed in broiler in America more over it has isolated from poultry and poultry products and it was of high antibiotic resistance (Fricke *et al*., 2009).

S. Kentucky is isolated from several species hasn't any signs of illness as cattle, poultry, poultry products, environment and domesticated dogs in the United States (Haley *et al*., 2016 a) S. Kentucky ST198 is responsible for several human cases who were travel to Middle East, Southeast Asia or Africa (LeHallo *et al*., 2011, 2013 a,b).

Screening by PCR based on 11 well known virulence genes was applied. The results showed that variable dissemination percent among *Salmonella* kentucky (table 2). The results indicated that only little or no variation was found for genes incorporated in SPIs and for the fimbrial marker, which is in accordance with (Huehn *et al*., 2010) and assure that virulence genes are widely distributed among Salmonella serovars.

The variety of virulence factors among *Salmonella* serovars has resulted in differences in their pathogenicity (Fluit, 2005). The detection of *inv*A gene in all the examined isolates is in agreement with previous reports in Egypt (Osman *et al*., 2013, 2014a, 2014b, Ahmed *et al*., 2016). and worldwide (Chuanchuen *et al*., 2010; Borges *et al*., 2013; Rowlands *et al*., 2014). The *invA* gene encodes for a protein in the inner and outer membrane, which is essential for the invasion of epithelial cells (Darwin and Miller, 1999). These studies described this gene as a marker for the molecular detection of *Salmonella* serotypes by PCR (Salehi *et al*., 2005).

The *inv*A gene, the *sopB*, *bcfC*, *avr*A,*ssa*Q and *mgt*C genes were present in the most of strains. On contrary, the *gip*A gene was absent from all *Salmonella* strains.

Based on the PCR with 11 most important virulence genes, the virulotyping results for tested *Salmonella* Kentucky strains show variable results (*sop*B (24\26), *avr*A, *bcf*C and *ssa*Q (23\26), *mgt*C (21\26), *sop*E and *sod*C (5\26), *sii*D (3\26), *spv*C (1\26), while no one carry *gip*A.

In our work *sop*B, *avr*A, *ssa*Q, *mgt*C, *bcf*C have the highest recorded Percent of tested virulence genes that's nearly similar to (Huehn *et al*., 2010, Osman *et al*., 2014b).

*avr*A gene was detected also in 88.4% of the isolates. The high frequency of this gene is only observed in serovars that have a potential to cause severe salmonellosis in humans (Borges *et al*., 2013).

The inclusion and reassortment of such prophage-associated virulence genes may help Salmonella to change its behavior adaptation and acquire new changes. Also fimbriae are responsible for adhesion of bacterium to the cells. They are a set of fimbrial determinants (including *bcf, agf, csg, fim, lpf, saf, stb, stf, and STM*4595) which is common between *Salmonella* serovars and responsible for colonization of host cells. (Huehn *et al*., 2009).

The *sop*B gene associated with prophages was found in 92.3% of the examined isolates. Different studies have also reported the detection of that gene in almost all the *Salmonella* isolates from food and human origin (Borges *et al*., 2013; Ahmed *et al*., 2016).

There are other genes on prophage may have a role in virulence as the prophages Gifsy1, 2, and 3, Fels-1 and 2, and *Sop*EF (Ehrbar and Hardt, 2005). The SPI-1 secreted effectors *Sop*E and *Sop*E2 act as guanine nucleotide-exchange-factors (GEFs) for the small GTPases Cdc42 and Rac (Thomson *et al*. 2004) in present study was detected by 19% which is nearly low as in (Osman *et al*., 2014b).

*Sod*C found in pathogenic Gram-negative and Gram-positive bacteria (Sanjay *et al.*, 2010). It is responsible for protecting the pathogens against superoxide radicals generated by inflammatory and phagocytic cells during infections has been emphasized, non-detection of *sod*C may be due to their low expression and/or the instability of the enzyme due to proteolysis (Sanjay *et al*., 2010).

The virulence plasmid gene *spv*C was detected in the lowest percent among other virulence genes (Huehn *et al*., 2010).

*gip*A was absent in all strains as found in (Osman *et al*., 2014). *gipA*, is stimulated when the bacteria colonize the small intestine, after infection takes place several genes are elicited due to bacterial growth in Peyer's patch in small intestine (Stanley *et al*., 2000).

This study shows that virulence genes are widely distributed among *Salmonella* Kentucky which may pose as potential risk for poultry and human infections. Virulence genes are located on transmissible genetic elements as transposons, plasmids or bacteriophages or pathogenicity islands (Hacker *et al*., 1997).

In conclusion, the presence of these entire virulence gene in *Salmonella Kentucky* explain the increase of rate of isolation of this serotype from human and animals allover the world.

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الكشف عن بعض جينات الضراوة للسالمونيلا كنتاكى المعزولة من الدواجن

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تنتشر السالمونيلا كنتاكي بشكل متزايد في جميع أنحاء العالم بين الإنسان والحيوانات وهى تشكل مشكلة كبيرة. ولذا تم تجميع عدد ستة وعشرون معزولة من السالمونيلا كنتاكي من عينات مختلفة فى خلال الفترة من ٢٠١١ حتى ٢٠١٦ ، منها اثنان وعشرين من الدجاج وأربعة من السمان. وفي هذا البحث تم فحص عدد ١١ من جينات الضراوة التالية (*inv*A, *avr*A, *ssa*Q, *mgt*C, *sii*D, *sop*B, *gip*A, *sod*C1, *sop*E1, *spv*C, *bcf*C) باستخدام تفاعل إنزيم البلمرة المتسلسل ، وقد وجدت هذه الجينات بالنسب الأتية: *inv*A 100% ، *sop*B (٩٢.٧٪) و *avr*A, *bcf*C 88.4% و *ssa*Q, *mgt*C 80.7% و *sop*E *sod*C (١٩.٢٪) و (*sii*D) (١١.٥٪) و (spvC) (٣.٨٪) بينما لا توجد أى معزولة تحمل الجين *gip*A . وتشير هذه النتائج لوجود جينات الضراوة في السالمونيلا كنتاكي مما يؤكد مدى الخطورة المتوقعة على الدواجن والصحة العامة.