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Molecular detection of virulence factors in some food poisoning bacteria isolated from chicken meat and giblet

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ABSTRACT

Many bacterial detection rapid methods developed including nucleic acid-based analysis which considered the most precise, sensitive, and famous method of detection. This study aimed to investigate the bacterial hygienic quality of some chicken meat and giblet with special concern of molecular detection of some virulence factors associated with some isolated food poisoning bacteria. *E. coli*, *Salmonella*, *S. aureus*, and *Y. enterocolitica* strains were isolated from commercial and home-reared chicken meat and giblet in Menoufiya Governorate, Egypt. Accurately, *stx1*, *stx2*, *eaeA*, *and hylA* genes were detected in 45.4, 63.6, 18.1, and 27.2% of the isolated *E. coli* strains, respectively. *invA*, *hilA*, and *fimH* genes were detected in 100, 71.4, and 85.7% of the examined Salmonella isolates, respectively. Regarding to the examined *Y. enterocolitica* isolates, *Inv* gene was detected lonely in 25%, while it was mixed with *ystA* gene in 75% of the examined isolates. Detection of enterotoxigenic *Staph. aureus* genes revealed detection of staphylococcal enterotoxins genes types SEA, and SEB genes in 20, and 10%; moreover, mixed SEA+SED, SEB+SEC producing genes were detected in 10% for each, respectively. The present results proved that PCR assay is helpful, rapid and accurate detection method. Strict hygienic measures during slaughtering and handling of chicken meat and giblet must be followed.

1. INTRODUCTION

Chicken is one of the domesticated birds reared for their meat consumption. Chicken meat is a good source of prime quality protein; but unfortunately, it may acquire several foodborne pathogens during different processing treatment. It is recorded that when it is contaminated, it can cause foodborne illness to the human consumers (Bhandari et al., 2013).

Live birds had been infected with unique microorganisms on their feathers, skin and intestinal tract. For this reason, the infection of chicken meat and giblet starts from the time of slaughtering, defeathering, evisceration, until the very last product storage and distribution (Capita et al., 2004). Poultry are recognized to harbor a big range of bacteria that are pathogenic to human being.

Enterobacteriacae, especially *E. coli* and Salmonella considered important food poisoning organisms; besides being involved as an indicator for possible fecal contamination (Synge, 2000). Their accumulation in poultry cuts and its products indicates lack of proper sanitation.

In recent years, *E. coli*, *Salmonella*, and *Staph. aureus* have become recorded as a serious foodborne pathogens and has been associated with numerous foodborne outbreaks, where *E. coli* includes a variety of different types that range from virulent commensal strains to highly pathogenic strains that cause variable degrees of infections in both humans and animals (Kaper et al., 2004); namely, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, and enteroaggregative *E. coli* (Gomez-Duarte, 2013). Shiga toxin–producing *E. coli* (STEC) can lead to sporadic cases and outbreaks that can cause several illnesses, such as hemolytic colitis (HC) and hemolytic uremic syndrome (HUS), following the onset of diarrhea.

In addition, Salmonella was contributed among the causes of worldwide foodborne pathogens. According to an estimation made in 2010, Salmonellae were involved in more than 80 million cases of foodborne gastroenteritis every year worldwide, of which 155,000 were fatal (Majowicz et al., 2010).

Regarding to *Staph. aureus*, recorded by Normanno *et al.* (2007) as the most pathogenic species of Staphylococci that is considered the 3rd most foodborne disease causing in the world, which essentially referred to its wide variety of enterotoxins production named Staphylococcal enterotoxins. Traditional most frequent SEs were recorded to be SEA to SEE; in addition, SEG to SEI, SER to SET may be detected with demonstrated emetic activity and gastrointestinal troubles.

Bolton *et al.* (2013) said that yersiniosis is a gastrointestinal infection caused by *Y. enterocolitica* which is considered the most prevalent gastrointestinal infection after Campylobacteriosis and salmonellosis in the industrial countries**.** It was estimated that *Y. enterocolitica* causes about 117,000 infected cases, 640 hospitalizations, and 35 deaths in the USA/year (CDC, 2019).

Ingestion of such foodborne pathogens is mainly incriminated in many food poisoning symptoms including gastroenteritis and sometimes systemic infections. The initial symptoms are dramatic diarrhea, which is sometimes accompanied by abdominal pain, nausea, vomiting,

headaches, chills, myalgia and variable-grades of fever (Ziprin and Hume, 2001). Detection of foodborne pathogens basing on traditional identification of microorganisms by their biochemical, morphological and immunological characteristics using selective culture media are time consuming and possibility of errors can occur in enumeration and sampling when microorganism present in low number in the sample. So, Methods based on nucleic acid detection, PCR (Polymerase Chain Reaction), identified as a powerful diagnostic method for the detection of pathogenic microorganisms; these techniques are specific, rapid, and sensitive in detection and identification of organisms comparing with other methods (Wang et al., 2007). Therefore, this study aimed to molecular detection of some virulence factors associated with some isolated food poisoning bacteria.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of forty bacterial isolates represented by 11 *E. coli* represented by serotypes (O_2:H_6, O_{26}:H_{11}, O_{55}:H_7, O_{78}, O_{91}:H_{21}, O_{111}:H_2, O_{119}:H_6, O_{124}, O_{128}:H_2 O_{153}:H_2 and O_{158}) strains, 7 Salmonella represented by (*S. Enteritidis*, *S. Kentucky*, *S. Larochelle*, *S. Molade*, *S. Papuana*, *S. Takoradi* and *S. Typhimurium* serotypes), 12 *Y. enterocolitica*, and 10 *Staph. aureus* isolates were investigated. Such pathogenic strains were isolated from different fresh chicken meat and giblet collected from home-reared (of 45 days old) and commercial chicken carcasses in Menoufiya governorate, Egypt; during the period of January to December 2018 and kept at -18^{O}C until molecular examination for detection of some virulence factors associated with them was performed.

2.2. The strains under examination were isolated according to:

- ISO 16649-2 (2001) for detection and isolation of *E. coli*; which were serologically identified according to Kok *et al.* (1996).
- ISO 6579 (2017) for isolation and identification of Salmonellae; which were serologically identified according to Kauffman – White scheme (Kauffman, 1974).
- ISO (6888-1:1999, A1:2003) for detection and isolation of *S. aureus*.
- ISO 10273 (2017) for detection of *Yersinia enterocolitica.*

2.3. Primer sequences of E. coli, Salmonella, Y. enterocolitica, and Staph. aureus virulence genes used for PCR identification system as follow in Tables (1 to 4). *E. coli* was examined for the presence of *stx1*, *stx2*, *eaeA* and *hylA* genes; while, Salmonellae were examined for the presence of *invA*, *hila* and *fimH* genes; furthermore, *Staph. aureus* was examined for the presence of SEs (A to D). Finally, *Y. enterocolitica* was examined for the presence of *inv* and *ystA* genes.

Table 1 Primer sequences of *E. coli* genes used for PCR identification system.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Mazaheri et al. (2014)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3	165	Fratamico et al. (1995)
hylA (R)	5′ CTTCACGTGACCATACATAT ′3		

Table 2 Primer sequences of Salmonellae genes used for PCR system

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
invA (F)	5′ GTGAAATTATCGCCACGTTCGGGCA ′3	284	Shanmugasamy et al. (2011)
invA (R)	5′ TCATCGCACCGTCAAAGGAACC ′3		
hilA (F)	5′ CTGCCGCAGTGTTAAGGATA ′3	497	Guo et al. (2000)
hilA (R)	5′ CTGTCGCCTTAATCGCATGT ′3		
fimH (F)	5′ GGA TCC ATG AAA ATA TAC TC ′3	1008	Menghistu (2010)
fimH (R)	5′ AAG CTT TTA ATC ATA ATC GAC TC ′3		

Table 3 Primer sequences of enterotoxin genes of *Staph. aureus*

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	Rall et al. (2008)
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3		
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
sec (R)	5′ AAATCGGATTAACATTATCC ′3		
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3		

Table 4 Primer sequences of *Y. enterocolitica* genes used for PCR identification.

Target gene	Primers	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
inv	YC1 (F)	5′CTGTGGGGAGAGTGGGGAAGTTTGG′3	570	Rasmussen et al. (1994)
	YC2(R)	5′GAACTGCTTGAATCCCTGAAAACCG ′3		
ystA	Pr2a (F)	5′ AATGCTGTCTTCATTTGGAGCA ′3	145	Ibrahim et al. (1997)
	Pr2c (R)	5′ ATCCCAATCACTACTGACTTC ′3		

2.4. DNA preparation from bacterial culture was performed according to Shah et al. (2009).

2.5. DNA amplification:

2.5.1. Amplification reaction of E. coli was performed according to Fagan et al. (1999).

2.5.2. Amplification of virulence genes of Salmonellae was performed according to Singh et al. (2013).

2.5.3. Amplification reaction of inv and ystA genes of Y. enterocolitica was performed according to Momtaz et al. (2013).

2.5.4. Amplification of enterotoxin genes of Staph. aureus was performed according to Mehrotra et al. (2000).

3. RESULTS

Table (5) showed the occurrence of virulence genes of Shiga-toxin producing *E. coli* strains where, *STX1, STX2, eaeA,* and *hylA* genes were detected in 45.4, 63.6, 18.1, and 27.2% of the examined strains, respectively. Fig. (1) showed the agarose gel electrophoresis bands proving the detection of *STX1* gene in *E. coli* O_{78}, O_{128} and O_{158} as shown in lanes (4, 9, and 11), respectively; lanes (1, 3, and 10) representing *E. coli* O_2, O_{55} and O_{153} as positive *E. coli* for *STX2* gene; lane (7) representing *E. coli* O_{119} as positive strain for both *STX1* and *STX2* genes; lane (5) representing *E. coli* O_{91} as positive strain for *STX1, STX2,* and *hlyA* genes; lanes (2, and 6) representing *E. coli* O_{26}, and O_{111} serotypes as positive for *STX1, STX2, eaeA* and *hlyA* genes. Finally, lane (8) representing *E. coli* O_{124} as negative *E. coli* strain for all *STX*1, *STX*2, *eaeA* and *hlyA* genes. Table (6) presented the incidence of the examined virulence genes in Salmonellae isolates, where *hilA,* and *fimH* genes were detected at an incidence of 71.4, and 85.7% in the examined isolates, respectively. While, *invA* was detected in 100% of examined strains. Moreover, fig. (2) showed the agarose gel electrophoresis results. Lanes (1, 2, 4, and 7) showed that *S. enteritidis, S. kentuckey, S. molade, and S. typhimurium* as positive strains for *invA, hilA* and *fimH* genes. Lane (3) showed that *S. larochelle* had both *invA* and *hilA* genes. Lanes (5, 6) representing *S. papuana* and *S. takoradi* as positive strains for *invA* and *fimH* genes.

Staphylococcal enterotoxin A, B, A+D, and B+C genes were detected in 20, 10, 10, and 10% in five isolates, while 50% of the examined *Staph. aureus* isolates showed absence of enterotoxins genes (-ve) as shown in table (7); furthermore, Fig. (3) shows the agarose gel electrophoresis reading proving the results of SEs (*SEA, SEB, SEC,* and *SED*) genes in the examined *Staph. aureus* isolates, where lanes 4 and 9 represented positive *Staph. aureus* strains for *SEA* gene; lane 2 as positive *Staph. aureus* strain for *SEB* gene; lane 7 as positive *Staph. aureus* strain for mixed *SEA* and *SED* genes; lane 10 as positive *Staph. aureus* strain for both *SEB* and *SEC* genes. Finally, five strains showed absence of enterotoxin genes as non-toxigenic strains as present in lanes 1, 3, 5, 6, and 8.

Table 5 Occurrence of virulence genes of Shiga toxin-producing *E. coli* strains isolated from chicken meat and giblets (n=11)

E. coli Serovars	*stx1*	*stx2*	*eaeA*	*hylA*
O_2:H_6	-	+	-	-
O_{26}:H_{11}	+	+	+	+
O_{55}:H_7	-	+	-	-
O_{78}	+	-	-	-
O_{91}:H_{21}	+	+	-	+
O_{111}:H_2	+	+	+	+
O_{119}:H_6	+	+	-	-
O_{124}	-	-	-	-
O_{128}:H_2	-	-	-	-
O_{153}:H_2	-	+	-	-
O_{158}	-	-	-	-
Total incidence*	45.4	63.6	18.1	27.2

* representing the incidence of occurrence in relation to total number of examined isolates (11). *Stx1*: Shiga-toxin 1 gene. *Stx2*: Shiga- toxin 2 gene. *EaeA*: intimin gene. *hylA*: haemolysin gene

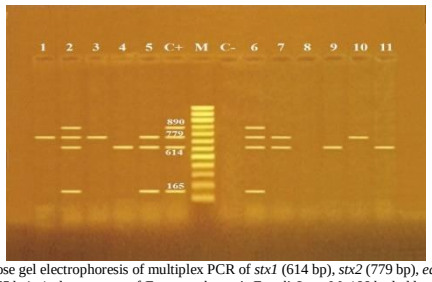

Fig. 1 Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) virulence genes of *Enteropathogenic E. coli*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1, stx2, eaeA* and *hlyA* genes. Lane C-: Control negative. Lanes 4 (O78), 9 (O128) & 11 (O158): Positive *E. coli* for *stx1* gene. Lanes 1 (O2), 3 (O55) & 10 (O153): Positive *E. coli* for *stx2* gene. Lane 7 (O119): Positive *E. coli* for *stx1* and *stx2* genes. Lane 5 (O91): Positive *E. coli* for *stx1, stx2* and *hlyA* genes. Lanes 2 (O26) & 6 (O111): Positive *E. coli* for *stx1, stx2, eaeA* and *hlyA* genes. Lane 8 (O124): Negative *E. coli* for stx1, stx2, eaeA and hlyA genes.

Table 6 Incidence of virulence genes of different *Salmonella* strains isolated chicken meat and giblets (n=7).

Salmonella Serovars	*invA*	*hilA*	*fimH*
S. Enteritidis	+	+	+
S. Kentucky	+	+	+
S. Larochelle	+	+	-
S. Molade	+	+	+
S. Papuana	+	-	+
S. Takoradi	+	-	+
S. Typhimurium	+	+	+
Total incidence*	100	71.4	85.7

* Representing the incidence of occurrence in relation to total number of examined isolates (7). *invA*: invasion A gene. *hilA*: hyper-invasive locus gene. *fimH*: fimbrial gene

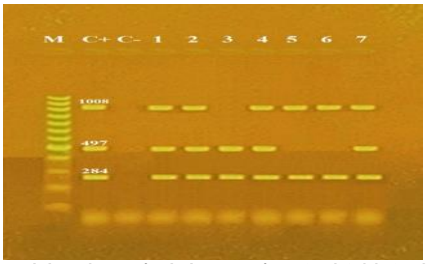

Fig. 2 Agarose gel electrophoresis of multiplex PCR of *invA* (260 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulence genes for characterization of Salmonella strains. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *S. Typhimurium* for *invA, hilA* and *fimH* genes. Lane C-: Control negative. Lanes 1 (*S. enteritidis*), 2 (*S. kentuckey*), 4 (*S. molade*) & 7 (*S. typhimurium*): Positive strains for *invA, hilA* and *fimH* genes. Lane 3 (*S. larochelle*): Positive strain for *invA* and *hilA* genes. Lanes 5 (*S. papuana*) & 6 (*S. takoradi*): Positive strains for *invA* and *fimH* genes.

Regarding to *inv* and *ystA* genes of *Y. enterocolitica* isolates, Table (8) showed that *inv* was detected alone in 25%, while it was mixed with *ystA* gene in 75% of the examined isolates. In addition, Fig. (4) showed presence of *inv* gene bands in lanes 4, 6, and 11; while both *inv* and *ystA* genes were detected in lanes 1, 2, 3, 5, 7, 8, 9, 10, and 12.

Table 7 Occurrence of enterotoxin genes of *S. aureus* strains isolated from chicken meat and giblets (n= 15 strains)

S. aureus enterotoxins	No.	%
A	2	20
B	1	10
A+D	1	10
B+C	1	10
-ve	5	50
Total	10	100

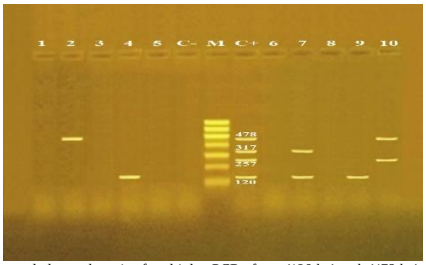

Fig. 3 Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) enterotoxin genes for characterization of *Staph. aureus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *sea, seb, sec* and *sed* genes. Lane C-: Control negative. Lanes 4 & 9: Positive *S. aureus* strains for *sea* gene. Lane 2: Positive *S. aureus* strain for *seb* gene. Lane 7: Positive *S. aureus* strain for *sea* and *sed* genes. Lane 10: Positive *S. aureus* strain for *seb* and *sec* genes. Lanes 1, 3, 5, 6 & 8: Negative *S. aureus* strains for enterotoxins

Table 8 Occurrence of virulence genes of *Y. enterocolitica* isolated from chicken meat and giblets (n=12)

Virulence genes	No.	%
inv + ystA	9	75
Inv	3	25
Total	12	100

inv: chromosomal invasion gene. *ystA*: Yersinia stable toxin gene

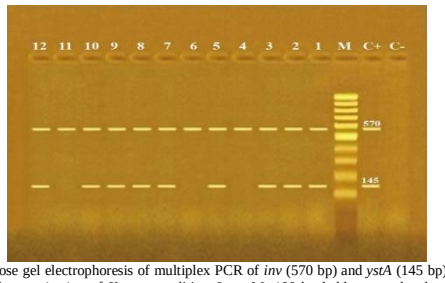

Fig. 4 Agarose gel electrophoresis of multiplex PCR of *inv* (570 bp) and *ystA* (145 bp) virulence genes for characterization of *Y. enterocolitica.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *Y. enterocolitica* for *inv* and *ystA* genes. Lane C-: Control negative. Lanes 1, 2, 3, 5, 7, 8, 9, 10 & 12: Positive *Y. enterocolitica* for *inv* and *ystA* genes. Lanes 4, 6 & 11: Positive *Y. enterocolitica* for *inv* gene.

4. DISCUSSION

Chicken meat is a prime source of white meat and protein of high biological value (Shedeed, 1999). Unfortunately, fresh chicken carcasses may host large number of foodborne pathogens from their feathers or the alimentary tract during slaughtering processes including the additional bacterial load from the environment, equipment and operator′s hands (Živković, 2001); which predisposing food poisoning especially with bacterial pathogens (Sodha et al., 2009). Therefore, rapid, sensitive, and accurate detectors such as PCR assays were developed (Hassan, 2012). Foodborne Enterobacteriacae bacteria such as *E. coli* and Salmonella are incriminated in many human diseases causing suppurative lesions, neonatal septicemia and meningitis (Collins et al., 1991). Between 2003 and 2012, 390 *E. coli* food poisoning outbreaks were encountered in the USA, resulting in 4,928 cases, 1,272 hospitalizations and 33 deaths (Heiman et al., 2015), while CDC (2020) estimated that *Salmonella* bacteria cause about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year, where food of animal origin is the main source for most of these illnesses.

Pathogenic *E. coli* infectivity is related to several virulence factors, such as intimin (*eaeA*), hemolysin (*hylA*), *STX*1, and *STX*2; *eaeA* and *hylA* genes responsible for the bacterium's adherence to the intestinal mucosa, and lyses erythrocytes, respectively, while *STX*1 and *STX*2 genes increase the intestinal motility and solution accumulations (Paton and Paton, 1998). These genes were reported to be the main factors associated with *E. coli* food poisoning which may lead to the occurrence of HC and HUS in humans in advanced cases (Sami and Roya, 2007).

Results of molecular detection of *E. coli* virulence genes represented by *STX*1, *STX*2, *eaeA*, and *hylA* genes in the examined isolates as mentioned in Table (5) and Fig. (1) are in agree with those recorded by Mohamed (2017), Abdallah (2018), Mustafa (2018), and El-Hanafy (2019) who detected *E. coli* virulence genes in their isolates from raw chicken meat samples.

In addition, several Salmonella specific virulence genes such as *invA, hila,* and *fimH* were recorded to take an important role in the pathogenicity have been identified; where in *S. Typhimurium* serovar, at least 80 different virulence genes have been identified (Baumler et al. 2000). Some genes are known to be involved in adhesion and invasion, like *fimH* (Duncan et al., 2005), *invA* (Galan et al., 1992), and other genes associated with toxin production.

Results of the detection of Salmonella virulence genes as mentioned in Table (6) and Fig. (2) were in agree with those recorded by Eissa (2017), Abd El-Halim (2017), Abdallah (2018), and El-Hanafy (2019) who detected different Salmonella virulence genes in their different Salmonella isolates such as *S. enteritidis*, *S. typhimurium* and *S. Papauna* which were isolated from different raw chicken meat products.

Regarding to *Staph. aureus* enterotoxins genes, Jørgensen *et al.* (2005) said that *Staph. aureus* produces many important virulence factors including SEs which were reported in more than 70% of *Staph. aureus* isolates. Staphylococcal enterotoxins (SEs) are responsible for diarrhea, vomiting and other symptoms associated with staphylococcal food poisoning.

The present results as demonstrated in table (7) and fig. (3) agreed with those recorded by Ahmed (2016), Abd El-Salam (2018), Gaafar (2018), Naguib (2017), and El-Hanafy (2019) who detected different SEs producing genes in their entero-toxigenic *Staph. aureus* isolates from raw chicken meat cuts, and chicken meat product samples.

Yersiniosis is an infection caused most often by eating raw or undercooked contaminated meat with *Y. enterocolitica* bacteria. It was estimated that *Y. enterocolitica* causes almost 117,000 illnesses, 640 hospitalizations, and 35 deaths in the United States every year, where children were infected more often than adults, and the infection is more common in the winter (CDC, 2016). Regarding to detection of *Y. enterocolitica* virulence genes as presented in Table (8) and Fig. (4), previous study conducted by Shabana (2015) reported detection of *ystA* gene in *Y. enterocolitica* strains isolated from raw chicken meat cut samples.

Compliance of the present results, with the previous reports proved that fresh chicken meat and giblet still have been exposed to several food poisoning bacterial sources; in addition, PCR is a good and reliable confirmatory diagnostic assay for virulence bacteria.

5. CONCLUSION

From the present results, it was concluded that polymerase chain reaction (PCR) can be useful, rapid, and confirmatory detector of a single copy virulence genes of pathogenic bacteria in chicken meat and giblet, and thus, it is recommended to be used to detect pathogenic bacterium in food rapidly.

CONFLICT OF INTEREST

No conflicts of interest.

6. REFERENCES

1. Appleford, M.R., Oh S., Oh N., Ong J.L., 2009. *In vivo* study Abd El-Halim, M.O. 2017. Public health importance of salmonellosis in Qualyobia province. Thesis, Master of Veterinary Medicine (Zoonosis), Benha University, Egypt.
2. Abd El-Salam, S.R. 2018. *Staphylococcus aureus* in broiler carcasses. Thesis, Master of Veterinary Medicine (Meat Hygiene), Benha University, Egypt.
3. Abdallah, R.R.M. 2018. Rapid detection of food borne pathogens in different food stuffs". Thesis, Master of Veterinary Medicine (Microbiology), Cairo University, Egypt.

4. Ahmed, Z.A. 2016. Detection of toxigenic *Staphylococcus aureus* in locally slaughtered chicken and beef in Luxor city by using of multiplex PCR. Thesis, Ph.D. of Veterinary Medicine (Meat Hygiene), South Valley Univ., Egypt.
5. Baumler, A.J., Tsolis, R.M., Heffron, F. 2000. Virulence mechanisms of Salmonella and their genetic basis. In: Salmonella in domestic animals. eds Wray, C., Wray, A. Wallingford, Oxford Shire, UK, CAB International, pp. 57–69.
6. Bhandari, N., Nepali, D.B., Paudyal, S. 2013. Assessment of bacterial load in broiler chicken meat from the retail meat shops in Chitwan, Nepal. International Journal of Infection and Microbiology, 2(3): 99-104.
7. Bolton, D. J., Ivory, C. and McDowell, D. 2013. A small study of *Y. enterocolitica* in pigs from birth to carcass and characterization of porcine and human strains". Food Control, 33(2): 521-524.
8. Capita, R., Alonso, C., Fernandez, M.D., Moreno, B. 2004. Microbiological quality of retail poultry carcasses in Spain. J. Food Protection, 64(12): 1961-1966.
9. CDC "Centers for Disease Control and Prevention" (2016): Information on this website focuses on Yersinia enterocolitica, which causes yersiniosis. https://www. cdc.gov/yersinia/. Accessed 10/3/2020.
10. CDC "Centers for Disease Control and Prevention" (2019): Yersinia enterocolitica (Yersiniosis): Questions and Answers. https://www.cdc.gov/yersinia/faq.html
11. CDC "Centers for Disease Control and Prevention" (2020): Salmonella. https://www.cdc.gov/salmonella/. Accessed 10/3/2020.
12. Collins, C.H., Lyne, P.M., Grange, J.M. 1991. Microbiological methods. Butter Worth, London, Boston, Toronto.
13. Dhanashree, B. and Mallya, S. 2008. Detection of shiga-toxigenic *Escherichia coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian J. Medical Research, 128: 271-277.
14. Duncan, M.J., Mann, E.L., Cohen, M.S., Ofek, I., Sharon, N., Abraham, S.N. 2005. The distinct binding specificities exhibited by enterobacterial Type 1 - Fimbriae are determined by their fimbrial shafts. J. Biology and Chemistry, 280: 37707–37716.
15. Eissa, M.O. 2017. Molecular characterization of Salmonella species isolated from some meat products. Thesis, Master of Veterinary Medicine (Bacteriology, Mycology and Immunology), Kafr Elsheikh University, Egypt.
16. El-Hanafy, A.R.A. 2019. Virulence factors associated with food poisoning bacteria in some beef and chicken meat products. Thesis, Master of Veterinary Medicine (Meat Hygiene), Benha University, Egypt.
17. Fagan, P., Hornitzky, M., Bettelheim, K., Djordjevic, S. 1999. Detection of Shiga-like toxin (*STX*1 and *STX*2), Intimin (eaeA), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR. Applied Environmental Microbiology, 65(2): 868–872.
18. Fratamico, P., Sackitey, S., Wiedmann, M., Deng, M. 1995. Detection of *Escherichia coli* O_{157}:H_7 by multiplex PCR. J. Clinical Microbiology, 33: 2188- 2191.
19. Gaafar, H.W. 2018. Demonstration of *Staph. aureus* in some meat products using PCR technique". Thesis, Master of Veterinary Medicine (Meat Hygiene), Benha University, Egypt.
20. Galan, J.E., Ginocchio, C., Costeas, P. 1992. Molecular and functional characterization of the Salmonella invasion gene *invA*: homology of *invA* to members of a new protein family. J. Bacteriology, 174: 4338-4349.
21. Gomez-Duarte, O.G., Romero-Herazo, Y.C., Paez-Canro, C.Z., Eslava-Schmalbach, J.H., Arzuza, O. 2013. Enterotoxigenic *Escherichia coli* associated with childhood diarrhoea in Colombia, South America Journal of infection in developing countries, 7(5): 372-381.
22. Guo X., Chen J., Beuchat, L., Brackett, R. 2000. PCR detection of *Salmonella enterica* serotype Montevideo in and on raw tomatoes using primers derived from *hilA*. Applied Environmental Microbiology, 66: 5248-5252.
23. Hassan, Z.H. 2012. Conventional and rapid detection of *Escherichia coli* and *Staphylococcus aureus* in some meat products. Thesis, Ph.D. of Veterinary Medicine (Meat hygiene), Menoufiya University (Sadat branch).
24. Heiman, K.E., Mody, R.K., Johnson, S.D., Griffin, P.M., Gould, L.H. 2015. *Escherichia coli* O_{157} outbreaks in the United States, 2003–2012. Emerged Infectious Diseases, 21(8): 1293–1301..
25. Ibrahim, A., Liesack, M., Griffiths, A., Robins-Browne, R. 1997. Development of a highly specific assay for rapid identification of pathogenic strains of *Yersinia enterocolitica* based on PCR amplification of the Yersinia heat-stable enterotoxin gene (yst). J. Clinical Microbiology, 35:1636-1638.
26. ISO "International Organization for Standardization" 10273:2017. Microbiology of the food chain — Horizontal method for the detection of pathogenic Yersinia enterocolitica.
27. ISO "International Organization for Standardization" 16649-2: 2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-D-glucuronide.
28. ISO "International Organization for Standardization" 6579-1:2017. Microbiology of the food chain Horizontal method for the detection, enumeration and serotyping of Salmonella - Part1: Detection of Salmonella spp.
29. ISO "International Organization for Standardization" 6888-1:1999, A1:2003. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium (includes amendment A1:2003).
30. Jørgensen, H.J., Mathisen, T., Lovseth, A., Omoe, K., Qvale, K.S., Loncarevic, S. 2005. An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. FEMS. Microbiol. Lett., 252(2): 267–272.
31. Kaper, J.B., Nataro, J.P., Mobely, H.L.T. 2004. Pathogenic *E. coli*. National Reviews Microbiology, 2(2): 123-140.
32. Kauffman, G. 1974. Kauffman white scheme. WHO, BD 172, L. Rev. 1. Acta Pathologica et Microbiologica Scandinavica, 61: 385.
33. Kok, T., Worswich, D., Gowans, E. 1996. Some serological techniques for microbial and viral infections. In: Practical Medical Microbiology, Collee, J., Fraser, A., Marmion, B. and Simmons, A. (Eds.), 14th Ed., Edinburgh, Churchill Livingstone, UK.
34. Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M. 2010. The global burden of non-typhoidal Salmonella gastroenteritis. Clinical Infectious Diesases, 50: 882-889.
35. Mazaheri, S., Ahrabi, S., Aslani, M. 2014. Shiga toxin-producing *Escherichia coli* isolated from lettuce samples in Tehran, Iran. Jundishapur J. Microbiology, 7(11): 1-6.
36. Mehrotra, M., Wang, G., Johnson, W. 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J. Clinical Microbiology, 38: 1032–1035.
37. Menghistu, H. 2010. Studies on molecular heterogeneity among *Salmonella gallinarum* isolates of poultry origin. Thesis, Master of Veterinary Medicine, Deemed Univ., IVRI, Izatnagar, Bareilly.
38. Mohamed, M.A.K. 2017. Challenge of multi drug-resistant *STX*1 harboring *E. coli* in meat and fast foods. Thesis, Master of Veterinary Medicine (Meat Hygiene), Benha University, Egypt.
39. Momtaz, H., Rahimian, M., Dehkordi, F. 2013. Identification and characterization of *Yersinia enterocolitica* isolated from raw chicken meat based on molecular and biological techniques. J. Applied Poultry Researches, 22: 137–145.
40. Mustafa, N.F. 2018. Studies on virulence genes of *E. coli* strains isolated from chickens intended for human consumption". Thesis, Master of Veterinary Medicine (Bacteriology, Mycology and Immunology), Mansoura University, Egypt.

41. Naguib, R.A. 2017. Detection of virulent genes responsible for *Staphylococcus aureus* enterotoxins production in chicken meat using PCR. Thesis, Ph.D. of Veterinary Medicine (Meat Hygiene), Benha Univ., Egypt.
42. Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N.C., Corrente, M., Parisi, A., Santagada, G., Firin, U.A., Crisetti, E., Celano, G.V. 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. International J. Food Microbiology, 115: 290-296.
43. Paton, A.W., Paton J.C. 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *STX* 1, *STX* 2, *eaeA*, Enterohemorrhagic *E. coli hlyA*, *rfb* O_{111} and *rfb* O_{157}. J. Clinical Microbiolpgy, 36: 598-602.
44. Rall, V., Vieira, F., Rall, R., Vieitis, R., Fernandes, A., Candeias, J., Cardoso, K., Araujo, J. 2008. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Vet. Microbiology, 132: 408–413.
45. Rasmussen, H., Rasmussen, O., Andersen, J., Olsen, J. 1994. Specific and detection of pathogenic *Yersinia enterocolitica* by two-step PCR using hot-start DMSO. Mol. Cell. Probes 8: 99–108.
46. Sami, M., Roya F. 2007. Prevalence of *Escherichia coli* O_{157}:H_7 on dairy farms in Shiraz, Iran by immunomagnetic separation and multiplex PCR. Iran J. Veterinary Researches, 4: 319-324.
47. Shabana, S.M. 2015. Identification and molecular analysis of *Yersinia enterocolitica* isolated from chicken meat samples". Thesis, Master of Veterinary Medicine (Microbiology), Alexandria University, Egypt.
48. Shah, D., Shringi, S., Besser, T., Call, D. 2009. Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, Pp. 369-389.
49. Shanmugasamy, M., Velayutham, T., Rajeswar, J. 2011. *Inv A* gene specific PCR for detection of Salmonella from broilers. Vet. World, 4 (12): 562-564.
50. Shedeed, N.A. 1999. Evaluation of microwave cooking of chicken meat. Thesis, Master of Agriculture, Cairo University.
51. Singh, S., Singh, H., Tewari, S., Prejit, N., Agarwal, R. 2013. Characterization of virulence factors among diverse Salmonella serotypes and sources. Advanced Animal Veterinary Science, 1(2): 69–74.
52. Sodha, S.V., Griffin, P.M., Hughs, J.M. 2009. Food born disease. In: Mandell GL, Bennett, JE, Dolin, R. (eds). Principles and practice of Infectious Disease .7th Ed., philadelphia, Elsevier Churchill Livingstone: Chap. 99, staphylococci: implications for our food supply. Anim. Health Res. Rev., 13: 157-180.
53. Synge, B.A. 2000. Verotoxin producing *E. coli*: A veterinary view. J. applied Microbiology, 88: 315-375.
54. Wang, L., Li, Y., Mustapha, A. 2007. Rapid and simultaneous quantification of *Escherichia coli* O_{157}:H_7, Salmonella and Shigella in ground beef by multiplex real-time PCR and immune-magnetic separation. J. Food Protection., 70(6): 1366-1372.
55. Ziprin, R.L., Hume, M.E. 2001. Human Salmonellosis: general medical aspects. In: Y.H. Hui, M.D. Pierson, and J.R. Gorham (eds.), Food borne Disease Handbook, 1st Ed., Bacterial Pathogens, Marcel Dekker, Inc., New York, NY. Pp. 285-321.
56. Živković, J. 2001. Meat hygiene and Technology. Veterinary and Sanitary Supervision of Animals for Slaughter and Meat. Part I. 2nd edition. M. Hadžiosmanović (Ed.). Faculty of Veterinary Medicine, University of Zagreb.