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 hlyA genes were detected in 45.4, 63.6, 18.1, and 27.2% of the isolated E. coli strains, respectively. invA, hlyA, and fimH genes were detected in 100, 71.4, and 38.5% 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 SEB+SED 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. Enterobacteriaceae, 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 SEL, 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 (O1:H6, O26:H11, O25:H7, O78, O41:H2, O111:H2, O116:H2, O124, O26:H2, O135:H2 and O138) strains, 7 Salmonella represented by (S. Enteritidis, S. Kentucky, S. Larochelle, S. Mollade, 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°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 Kaufmann – White scheme (Kaufmann, 1978).
- 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 hlyA 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 |
|-------------|-----------------------------------|-------------------|------------|
| stx (F)     | 5′ ACACTGAGATGCTACGAGTG ′3       | 614               | Dhanashree and Mallya (2008) |
| stx (R)     | 5′ CTGAAATCCCCCTTCAATTAG ′3      | 779               |           |
| Stx2 (F)    | 5′ CCATGCAACGGCAGACGGTT ′3       |                   |           |
| Stx2 (R)    | 5′ CCGTCGACTGAGACGCTTG ′3        |                   |           |
| eaeA (F)    | 5′ GTGGCGAATACTGCGGACGACT ′3     | 890               | Matecheri et al. (2014) |
| eaeA (R)    | 5′ CCCATTCTCTTTTTCACCGTCG ′3     |                   |           |
| hylA (F)    | 5′ AGCAAGTGGTGGTTTATTCTGG ′3     | 165               | Fritamico et al. (1995) |
| hylA (R)    | 5′ CTCTACGTCGACACATCATT ′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′ CTGAAATTTATCAGCAGCTCCTGGGA ′3 | 284               | Shambumaaamy et al. (2011) |
| invA (R)    | 5′ TCTGCCACCGTAAGAGAACCC ′3      |                   |           |
| hlaA (F)    | 5′ CTGCGCAGTGTATAGAGATA ′3       | 497               | Gao et al. (2000) |
| hlaA (R)    | 5′ CTGTGCCCTAATGCGAGT ′3         |                   |           |
| fimH (F)    | 5′ CGA TCC ATG AAA ATA TAC TC ′3 | 1008              | Menghista (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′ TGCGAAACCGGTAAAGAAAACGA ′3     | 120               |           |
| seA (R)     | 5′ GAACCTTCCATCATACAAAAAC ′3      |                   |           |
| seB (F)     | 5′ TGCTAATCAACTGIAACAAAGC ′3      | 478               | Rall et al. (2008) |
| seB (R)     | 5′ CCCTGACTCTTATAAGTGCC ′3       |                   |           |
| sec (F)     | 5′ GACATAAAAGCTAGGAAGA ′3        | 257               |           |
| sec (R)     | 5′ AAAAGTGAATACAGATTACCC ′3      |                   |           |
| sed (F)     | 5′ CATGGTGGAGTAAATCTCCCTC ′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′ CTCTGAGGGAGATGGGGGAAATTTGGG ′3 | 570               | Rasmussen et al. (1994) |
|             | YC2(R)  | 5′ GAACCTTGAATACCGGAAAACG ′3       |                   |           |
| ystA        | PrA (F) | 5′ AAGTGCCTTTACTTGGAGGCA ′3       | 145               | Ibrahim et al. (1997) |
|             | PrA (R) | 5′ ATCCCAATACACTGACTGCTT ′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. (1990).

2.5.2. Amplification of virulence genes of Salmonella 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 Montaz 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 hlyA 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 O103, O128 and O158 as shown in lanes (4, 9, and 11), respectively; lanes (1, 3, and 10) representing E. coli O2, O4 and O153 as positive E. coli for STX2 gene; lane (7) representing E. coli O119 as positive strain for both STX1 and STX2 genes; lane (5) representing E. coli O61 as positive strain for STX1, STX2, eaeA, and hlyA genes; lanes (2, and 6) representing E. coli O32 and O111 as positive strains for STX1, STX2, eaeA and hlyA genes. Finally, lane (8) representing E. coli O24 as negative E. coli strain for all STX1, STX2, eaeA and hlyA genes. Table (6) presented the incidence of the examined virulence genes in Salmonella isolates, where hla, 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. kentucky, S. molade, and S. typhimurium as positive strains for invA, hla, and fimH genes. Lane (3) showed that S. larochei had both invA and hla genes. Lanes (5, 6), representing S. pupana 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 both SEB and SEC genes. Finally, five strains showed absence of enterotoxin genes as non-toxicogenic 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=15)

| E. coli Serovars | stk1 | stk2 | eaeA | hlyA |
|------------------|------|------|------|------|
| O103             | +    | -    | -    | -    |
| O128             | +    | +    | -    | -    |
| O158             | +    | +    | +    | +    |
| O4               | +    | +    | -    | -    |
| O153             | +    | +    | -    | -    |
| O111             | +    | +    | -    | -    |
| O32              | +    | +    | +    | +    |
| O2               | +    | +    | -    | -    |
| O61              | +    | +    | -    | -    |
| O24              | +    | +    | -    | -    |
| O119             | +    | +    | -    | -    |

Total incidence* 45.4 63.6 18.1 27.2

* Representing the incidence of occurrence in relation to total number of examined isolates (15).

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

| Salmonella Serovars | stk1 | stk2 | eaeA | hlyA |
|---------------------|------|------|------|------|
| S. enteritidis      | +    | +    | -    | -    |
| S. kentucky         | +    | +    | -    | -    |
| S. molade           | +    | +    | +    | +    |
| S. typhimurium      | +    | +    | -    | -    |

Total incidence* 100 71.4 85.7

* Representing the incidence of occurrence in relation to total number of examined isolates (7).

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 |
| -                      | 8   | 53 |
| Total                  | 10  | 100|

Fig. 1 Agarose gel electrophoresis of multiple PCR of stk1 (614 bp), stk2 (779 bp), eaeA (490 bp) and stx (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 stk1, stk2 and hlyA genes. Lane C-: Control negative. Lanes 1 (O79), 9 (O128) & 11 (O158): Positive E. coli for stk1 gene. Lanes 1 (O22), 3 (O55) & 10 (O131): Positive E. coli for stk2 gene. Lane 7 (O119): Positive E. coli for stk1 and stk2 genes. Lane 5 (O91): Positive E. coli for stk1, stk2 and hlyA genes. Lane 2 (O26) & 6 (O111): Positive E. coli for stk1, stk2 and hlyA genes.

Fig. 2 Agarose gel electrophoresis of multiplex PCR of stk1 (280 bp), hlyA (481 bp) and stx (1085 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 stk1, hlyA and stx genes. Lane C-: Control negative. Lanes 1 (S. enteritidis), 2 (S. kentucky), 4 (S. molade) & 7 (S. typhimurium): Positive strains for stk1, hlyA and stx genes. Lane 3 (S. larochei): Positive strain for stk1 and hlyA genes. Lanes 5 (S. pupana) & 6 (S. takoradi): Positive strains for stk1 and hlyA genes.

Fig. 3 Agarose gel electrophoresis of multiplex PCR of stk1 (120 bp), stk2 (478 bp), stk2 (257 bp) and stx (157 bp) enterotoxin genes for characterization of Staph. aureus. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for sea, sec and sed genes. Lane C-: Control negative. Lanes 4 & 9: Positive S. aureus strains for sea gene. Lane 5: Positive S. aureus strain for sec gene. Lane 6: Positive S. aureus strain for sed gene. Lane 7: Positive S. aureus strain for stk1, stk2 and stk3 genes. Lane 8: Positive S. aureus strain for stk1, stk2, eaeA and hlyA genes.
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 (Zhivković, 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 Enterobacteriaceae 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 (hlyA), STX1, and STX2; eaeA and hlyA genes responsible for the bacterium’s adherence to the intestinal mucosa, and lyses erythrocytes, respectively, while STX1 and STX2 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 STX1, STX2, eaeA, and hlyA 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, bila, 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. Papauana 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 enterotoxigenic 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.

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