Sequencing of \textit{bcfC} Gene of \textit{Salmonella} Typhimurium Isolated from Ducks in Egypt

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ABSTRACT

The main objective of this study was to applying \textit{bcfC} gene sequence of \textit{Salmonella} Typhimurium recently isolated from ducks to give insight into the source and origin, molecular epidemiology, disease pattern of \textit{Salmonella} Typhimurium in Egyptian duck farms. Out of 75 fecal swab samples, 15 (20\%) local field isolates were detected and confirmed phenotypically by culturing, gram staining, biochemically and serologically to be \textit{Salmonella} Typhimurium. The PCR amplification with \textit{bcfC} gene-specific primers was conducted with genomic DNA, which revealed a product with the approximate size of 467 bp. The \textit{bcfC} gene was found in 7 (46.6\%) isolates of \textit{Salmonella} Typhimurium. Phylogenetic and partial gene sequence analysis of \textit{bcfC} gene of \textit{Salmonella} Typhimurium showed clear clustering of Egyptian isolates of \textit{Salmonella} Typhimurium and different \textit{Salmonella} strains uploaded from GenBank. Sequence identities between the isolated Egyptian strain and different \textit{Salmonella} Typhimurium strains from GenBank revealed 99.8-100\% homology. Open reading frame (ORF) analysis of \textit{Salmonella} typhimurium \textit{bcfC} gene using NCBI tool and ORF analysis of \textit{bcfC} gene protein translation using ExPasy (SIB Bioinformatics Resource Portal) indicated all open reading frames of a specified minimum size in a sequence of (453 bp). The 3 conserved domains region in the nucleotide sequence were PapC N-terminal domain (107-394bp), PRK15193 outer membrane usher protein (56-424bp), and FimD Outer membrane usher protein FimD/PapC (cell motility, extracellular structures, 56-424bp). The PapC N-terminal domain was a structural domain found at the N-terminus of \textit{S. typhimurium} PapC protein and had a central role in the pilus assembly chaperone usher system (CUP). Amino acids alignment report of the sequenced 415 amino acid of \textit{Salmonella} Typhimurium \textit{bcfC} gene showed great homology between the Egyptian \textit{Salmonella} Typhimurium strain and the different \textit{Salmonella} strains uploaded from GenBank. Nucleotide alignment report of the sequenced \textit{Salmonella} Typhimurium \textit{bcfC} gene at (417bp) demonstrated great homology between the Egyptian \textit{Salmonella} Typhimurium strain and the different \textit{Salmonella} strains uploaded from GenBank. In conclusion, the Egyptian \textit{Salmonella} Typhimurium isolate was related to the common sequence types isolated from humans and bovine-based products across the world especially in the United Kingdom, USA, Ireland, and México. Most of the duck farms from which we isolated the Egyptian \textit{Salmonella} Typhimurium isolates were located in the same geographical area of cattle farms in addition to the duck farms lacked the requirements of biosecurity, which could facilitate the circulatory transmission of salmonella strains between the human beings and other animal farms, including duck farms. Moreover, the PapC N-terminal conserved domain can be used as a vaccine target for vaccine production against \textit{S. typhimurium}. A PapC N-terminal conserved domain was a central conserved domain encoded by \textit{bcfC} gene of \textit{S. typhimurium}. A PapC N-terminal conserved domain can be used as a vaccine target for vaccine production against \textit{S. typhimurium}.

Keywords: bcfC gene, Conserved domain, Duck, GenBank, ORF, Phylogenetic tree, \textit{Salmonella} Typhimurium, Sequencing.

INTRODUCTION

\textit{Salmonella} infections are considered one of among the foremost major problems within the poultry industry. \textit{Salmonella} Typhimurium has been regarded to be frequently related to disease in numerous species, including humans, livestock, domestic fowl, rodents, and birds. Therefore, \textit{Salmonella} Typhimurium is described as the prototypical broad-host-range serotype (Rabsch et al., 2002). \textit{Salmonella typhimurium} has been found in 60\% of poultry carcass (Mann and McNabb, 1984) and is responsible for 93\% of the \textit{Salmonella} infections in ducklings (Badr and Nasef, 2016). \textit{Salmonella} Typhimurium has been isolated from 40\% of hatchlings and 1\% of older ducklings in Taiwan, although clear host species specific differences have also been detected. 12 Salmonella has been previously isolated from imported day old ducklings in Brazil and also the USA (Ribeiro et al., 2006 and Gaffga et al., 2012). Because the prevalence of Salmonella in duck products poses a risk to human populations, an urgent need exists to research the prevalence, disease risk to human populations, and also the global epidemiology of Salmonella serovars and specific clones. This information could also be wont to address Salmonella risk and promote evidence-based interventions in global public health (Osman et al., 2014).
Pili (fimbriae) play a central role in bacterial colonization and pathogenesis (Li and Thanassi, 2009). Fimbriae are proteinaceous extracellular structures and play a distinct role in adhesion, a major initial step for colonization and entry into host cells. Fimbriae have also referred to as to play a central role in interactions with macrophages, intestinal persistence, biofilm formation and bacterial aggregation in Salmonella serovars (LeDeboer et al., 2006). The fimbrial gene (bcfC) is located on a fimbrial structure and play a vital role in attachment and cell invasion of Salmonella typhimurium (Huehn et al., 2010). Fimbrial gene bcfC is widely distributed among Salmonella, these data are according to the essential functions of adhesion factors for the attachment and internalization processes that occur during pathogenesis (Borriello et al., 2012).

bcfC is fimbrial usher protein consists of three functional domains which are PapC N-terminal domain, PRK15193 outer membrane usher protein and FimD outer membrane usher protein FimD/PapC. PapC (pyelonephritis-associated pilus C) is an integral outer membrane usher protein that forms an assembly platform for pilus biogenesis, PapC has five functional domains, all of which are required for pilus biogenesis, It's a 24-stranded β-barrel transmembrane domain that permits translocation of the polymerized pilus fiber across the outer membrane and 4 globular domains: a periplasmic N-terminal domain (NTD), two periplasmic C-terminal domains (CTD1 and CTD2), and a plug domain (Plug) (Henderson et al., 2011 and Phan et al., 2011). The usher PapC N-terminal functional unit represents primary binding site for chaperone-usher formation (Ng et al., 2004; Nishiyama et al., 2005 and Li et al., 2010).

Therefore, the main aim of this study is applying genetic sequencing and phylogenetic analysis of bcfC gene by using bioinformatics approach to explore more information about bcfC protein and to give insight about the source and origin, molecular epidemiology, disease pattern of Salmonella Typhimurium in Egyptian duck farms. Also, identification of highly conserved domains in Salmonella Typhimurium bcfC gene sequences for vaccine designing production against Salmonella typhimurium.

MATERIALS AND METHODS

Ethical approval

No ethical approval was obtained from the Institutional Animal Ethics Committee because no invasive procedure was performed on the animals. However, this study was conducted in accordance to the Institutional Animal Ethics of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abbasia, Cairo, Agricultural Research Center (ARC).

Samples collection

Totally, 75 fecal swabs were collected that contained 30 from apparently healthy ducks and 45 from diseased ducks, these were collected from five duck farms in Qaluobia, Sharkia and Monofia governorates of Egypt.

Isolation of Salmonella Typhimurium

It was carried out according to methods described by ISO6579 (2002)

Identification of Salmonella Typhimurium

Microscopic examination

Suspected colonies were Gram's stained and microscopically examined according to methods described by Quinn et al. (2002).

Biochemical identification

Biochemical identification was performed on isolated organisms by using the Analytical Profile Index 20E (API 20E) system (Nucera et al., 2006).

Serological identification

Salmonella culture serotyping was carried out according to methods described by Kauffmann-White typing scheme (Popoff, 2001).

Molecular identification

DNA was extracted using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer’s instructions with modifications. PCR was performed on extracted DNA by using specific primer (Table 1) supplied from Metabion (Germany) to amplify bcfC gene according to Huehn et al. (2010). PCR was performed in a 25μl reaction containing 12.5 μl of Emerald Amp GT PCR Master Mix (Takara, Japan), 1 μl of each primer of 20 p/mol concentrations, 4.5 μl of water and 6 μl of DNA template, using an Applied Biosystems 2720 Thermal Cycler. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 μl of the products was loaded in each gel slot. A100 base pair (bp) DNA Ladder (Qiagen, Germany, GmbH) was utilized to determine the fragment sizes. The gel was photographed by means of a gel documentation system (Alpha Innotech, Biometra).
Table 1. Primer sequences, target gene and amplicon size

| Microorganism       | Gene | Primer sequences (5'-3') | Amplified segment product (base pair) | Reference     |
|---------------------|------|--------------------------|--------------------------------------|---------------|
| Salmonella Typhimurium | bcfC | F-5'-accagagacattgcctc c-3' | 467                                   | Huehn et al., (2010) |

Open reading frame analysis

The NCBI tools website was carried out for Open reading frame analysis (ORF) analysis of bcfC gene sequence of Salmonella Typhimurium (453 bp). ExPASY-Translate Tool-SIB Bioinformatics Resource Portal was used for ORF analysis of bcfC gene sequence of Salmonella Typhimurium (453 bp) (https://web.expasy.org/translate/).

Conserved domain Search

NCBI Search Tool was performed conserved domain analysis of the bcfC protein sequence.

Phylogenetic, amino acids and nucleotide sequence analysis of bcfC gene of Salmonella Typhimurium

It was performed in Elim biopharmaceuticals, Germany. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis was initially performed according to standard methods described by Altschul et al. (1990). A comparative analysis of nucleotide and deduced amino acids sequences was performed using the CLUSTALW multiple sequence alignment program, version 1.83 of Mega Align module of Lasergene DNA Star software in accordance to methods designed by Thompson et al. (1994) and phylogenetic analysis was performed using neighbor joining in MEGA6 (Tamura et al., 2013).

RESULTS AND DISCUSSION

Isolation and identification of Salmonella Typhimurium field isolates

Out of 75 fecal swab samples, 15 isolates were confirmed phenotypically, biochemically and serologically to be Salmonella Typhimurium in a prevalence of 20% (15/75). These finding agree with Osman et al. (2014) (18.5%) and Typhimurium (453 bp). ExPASY-Translate Tool-SIB Bioinformatics Resource Portal was used for ORF analysis of bcfC gene sequence of Salmonella Typhimurium (453 bp) (https://web.expasy.org/translate/).

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IG-like domains (each about 80 residues long) at the C-terminus (CTD1 and CTD2 (Phan et al., 2011). Interaction between the NTD and Plug domains is essential step for usher gating. A conserved and immunogenic domain considered as a unique target for various vaccine development against Salmonella (Jha et al., 2015; Singh et al., 2017).

Phylogenetic and partial gene sequence analysis of bcfC gene of Salmonella Typhimurium that was generated using neighbor joining in MEGA6 (Figure 6), showed three major clusters or branches, one representing the Egyptian Salmonella Typhimurium strain isolate with CPO22491.1, LT795114.1, CPO22497.1, LT571437.1, CPO14975.1, CPO14358.1, CPO22658.1, CPO18657.1, CPO24619.1, LN999997.1, CPO14356.1, CPO11233.1, CPO14977.1, the second cluster for CPO16754.1, CPO22003.1, CPO18659.1, CPO18655.1, CPO18635.1, CPO17232.1, CPO18633.1, CPO19383.1, CPO15526.1, CPO15524.1, CPO18661.1, CPO18651.1, CPO18648.1, and the third one for AF129435.1 and AF130422.1.

Nucleotide sequence distance of Salmonella Typhimurium bcfC virulence gene (figure 7) was created by the Mega Align module of laser gene DNA star. Sequence identities between the isolated Egyptian strain and different Salmonella Typhimurium strains uploaded from GenBank revealed that 99.8% to 100% homology. Nucleotide sequence analysis of bcfC virulence gene of the Egyptian isolated strain showed 100% nucleotide identity with the American Salmonella enterica subsp. enterica serovar Typhimurium strain CDC 2009K-1640 (accession No.CP014975), the American Salmonella enterica subsp. enterica serovar Typhimurium strain USDA-ARS-USMARC-1896 (accession No.CP014977) by Nguyen et al. (2016), the Irish Salmonella enteric subsp. enterica serovar Typhimurium strain SL1344RX (accession No.CP011233) by Fitzgerald et al. (2015), the Mexican Salmonella enterica subsp. enterica serovar Typhimurium strain YU15 (accession No.CP014358) and Salmonella enterica subsp. enterica serovar Typhimurium strain YU15-SO2 (accession No.CP014356) by Silva et al. (2016).

In this study the Egyptian Salmonella Typhimurium isolate was distributed into common sequence types isolated from humans and bovine-based products across the world especially in the United Kingdom, USA, Ireland and México. Most of the duck farms from which we isolated the Egyptian Salmonella Typhimurium isolates were located in the same geographical area of cattle farms in addition to these farms lacked the requirements of biosecurity, which facilitates the circulatory transmission of Salmonella strains between human and animal farms to duck farms, present results agree with Murgia et al. (2015); Ktari et al. (2016) and Yang et al. (2019). Wang et al. (2020) recorded that the relatively high frequency of invasive infection of Salmonella in commercial meat-type duck flocks may largely relate to semi-open rearing systems that lack effective biosecurity, the majority of S. Typhimurium isolates were grouped into ST19 (63.89%), the most common sequence types isolated from humans and animal-based food products across the world. Perhaps circulatory transmission generated between contaminated poultry meat and human beings.

So, this study concluded that it is possible that the realistic explanation for the existence of similar strains of salmonella typhimurium isolated from duck farms and cows farms and strains isolated from the human host is the absence of disinfection, sterilization operations and the absence of health requirements by farm workers in addition to that the duck farms were located in the same geographical area of bovine farms. Amino acids alignment report of the sequenced 415 amino acid of Salmonella Typhimurium bcfC gene showed (figure 5) great homology between the Egyptian strain and the different Salmonellae strains from GenBank. On the other hand, nucleotide alignment report of the sequenced 417bp of Salmonella Typhimurium bcfC gene showed (figure 8) high identity between the Egyptian strain and the different Salmonellae strains from GenBank.

**Figure 1.** Agarose gel showing PCR-amplified product of bcfC virulence gene of Salmonella Typhimurium isolated from ducks. Lanes (1, 4, 8, 9, 10, 12, and 14): samples positive for bcfC gene (467 bp), Lane (pos.): positive control, Lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker).
Figure 2. Open reading frame analysis of \textit{bcfC} gene nucleotide sequence

Figure 3. Open reading frame analysis of \textit{bcfC} gene protein translation using ExPasy (SIB Bioinformatics Resource Portal) showed all ORFs. The frame 2 is the longest one.

Figure 4. Conserved domains exist within the family region
Figure 5. Amino acids alignment of *bcfC* virulence gene of Egyptian isolated strain *Salmonella Typhimurium* and different *Salmonella Typhimurium* strains retrieved from GenBank using CLUSTALW multiple sequence alignment program version 1.83 of MegAlign module of Lasergene DNASTAR.

Figure 6. Phylogenetic tree for *Salmonella Typhimurium* *bcfC* virulence gene partial nucleotide sequences that was generated using neighbor joining in MEGA6, showing clear clustering of the Egyptian isolated strain (marked with red color) and different *Salmonella Typhimurium* strains uploaded from GenBank.
Figure 7. Nucleotide sequence distance analysis of bcfC virulence gene of Egyptian isolated strain and other Salmonella Typhimurium strains from GenBank.

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Figure 8. Nucleotides alignment of \( bcfC \) virulence gene sequence of Egyptian strain of \textit{Salmonella} Typhimurium and different \textit{Salmonella} Typhimurium strains retrieved from GenBank using CLUSTALW multiple sequence alignment program version 1.83 of MegAlign module of Lasergene DNASTAR.

**DECLARATIONS**

**Author's contributions**

Abeer Saad El-Maghraby designed the idea and concept of the review article, planned the study and Abeer Saad El-Maghraby, Abeer Mwafy and Hala Ahmed Al-Sawy designed and performed study design. All authors shared in writing, and approved the final version of manuscript.

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**Competing interests**

The authors declared that no competing interests exist.

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