Advanced Studies on Virulence Genes of *Salmonella* and *Shigella* species Isolated from Milk and Dairy Products

Gamal A. M. Younis¹, Rasha M. Elkenany¹* and Wesam S. Abd-Elmoati¹

¹Department of Bacteriology, Mycology, and Immunology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.

Authors’ contributions

This work was carried out in collaboration between all authors. Author GAMY designed the study, performed the statistical analysis and wrote the protocol. Authors RME and WSA wrote the first draft of the manuscript and managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

*Salmonella* and *Shigella* species are the main health problem in various portions of the world. This study gave rise to detect and enumerate *Salmonella* and *Shigella* species with detection of virulence genes by PCR in randomly collected raw milk and dairy products (ice cream, cheese, yoghurt, rice with milk and cream) from different vendors of village and dairy farms in Mansoura Governorate, Egypt during October 2016. A total of 24 (9.6 %) isolates from 250 samples (raw milk and dairy products) were recognised as *Salmonella* 6.4 % (16/250) and *Shigella* 3.2 % (8/250) species with their high prevalence in raw milk. Amongst serotypes of *Salmonella* species: S. Typhimurium 37.5 % (6/16), S. Enteritidis, S. Tsevie 18.75 % (3/16 each) and other serovars 25 % (4/16). Additionally, the identified *Shigella* species (8/250) were S. dysenteriae 50 % (4/8), S. flexneri 25 % (2/8) and S. sonnei 25 % (2/8). The average of total viable count of samples positive for *Salmonella* and *Shigella* in raw milk and dairy products was $4.47 \pm 0.97 \log_{10}^{10}$ CFU/ml or gm and $4.27 \pm 1.01 \log_{10}^{10}$ CFU/ml or gm.

*Corresponding author: E-mail: dr_rashavet22@yahoo.com;
that result in abdominal pain and destruction, and fibrosis of the colonic mucosa, inflammation, ulceration, pathogenesis of shigellosis includes and middle income countries world, is one of the major causes of morbidity dysentery. Shigella dysenteriae, S. flexneri, S. sonnei Shigella[10, 11] identified from mostly all the has been considered a universal genetic marker triggers the pathogen to invade the host cell and for detection of gene is one of the virulence encoding identification. The chromosomally located application. The bacterial contamination of milk and dairy products was largely due to the human factor and unhygienic conditions application. The presence of the pathogenetic bacteria in milk considers main public health concerns [1,2]. The environment and food were primarily contaminated with Salmonella and Shigella by the faecal wastes of the infected animals and humans [3,4] as Salmonella and Shigella colonised mostly in the gastrointestinal tract [5,6].

Salmonella Enterica cause "salmonellosis" which is one of the most common food-borne disease [7] and associated with a major economic productivity loss in the food and animal industries [8]. Salmonellosis is zoonotic unusually contagious disease, usually self-limiting infection [9]. There are arrays of virulence factors that are responsible for the pathogenic ability of Salmonella act in tandem and eventually manifest in the typical symptoms of salmonellosis. In latest years, PCR is commonly applied for disease diagnosis and bacteria identification. The chromosomally located invA gene is one of the virulence encoding-gene used for detection of Salmonella genus and associated with Salmonella pathogenicity islands (SPIs). It triggers the pathogen to invade the host cell and has been considered a universal genetic marker identified from mostly all the Salmonella serovars [10,11].

Shigella is categorised into four serogroups: S. dysenteriae, S. flexneri, S. sonnei and S. boydii [12]. Shigella species have global human health problem by causing: "Shigellosis or bacillary dysentery". Shigellosis, endemic throughout the world, is one of the major causes of morbidity and mortality, particularly amongst children in low and middle income countries [13,14]. The pathogenesis of shigellosis includes inflammation, ulceration, hemorrhage, tissue destruction, and fibrosis of the colonic mucosa, that result in abdominal pain and diarrhea/dysentery; in some cases infertility and endometriosis also have been documented [3,15]. Shigella strains have a lot of virulence attributes that are related to their pathogenicity such as invC, ipaH and virA genes. The invC gene can identify Shigella at the genus level [16]. The ipaH gene, the invasion plasmid H, is species-specific gene and presents in all Shigella strains [17]. The virA gene has been implicated in invasion and intercellular spreading [18].

For reduction of milk and dairy products contamination and diseases caused by Salmonella and Shigella infections, the present study was conducted to throw light on the occurrence and enumeration of Salmonella and Shigella species in randomly collected raw milk and dairy products samples and uses of molecular methods for detection of virulence genes of Salmonella and Shigella species in Egypt.

2. MATERIALS AND METHODS

2.1 Sampling

A simple random method was adopted to collect a total of 250 raw milk and dairy product samples (150 raw milks, 37 ice creams, 30 kareish cheeses, 20 yogurts, 5 rice with milks, 8 creams) from different vendors of village and dairy farms in Mansoura Governorate, Egypt during October 2016. The samples were maintained on ice box until transported to the laboratory and processed within 1 h of collection.

2.2 Isolation and Identification of Salmonella and Shigella species

All samples (raw milk and dairy products) were prepared as 25 ml or gm added to 225 ml of sterile buffered peptone water (Oxoid), and then incubated at 37°C for 6 h. One milliliter of prepared culture was aerobically enriched in
tryptone soya broth (Oxoid) at 37°C for 24 h for *Shigella* and Rappaport Vassiliadis (RV) broth (Oxoid) at 37°C and 41°C for 24 h for *Salmonella*. A single drop of prepared pre-enrichment specimen was inoculated with streaking onto Salmonella-Shigella agar (S-S agar), Xylose Lysine Deoxycholate agar (XLD) and MacConkey’s agar (MAC) (Oxoid) and then incubated at 37°C for 24 h. Non-lactose fermenting colonies were picked up from culture plates and biochemically tested (triple sugar iron agar, indole, urease and Simmon’s citrate agar tests). Serological confirmation of suspected colonies of *Salmonellae* was carried out according to Kauffman White scheme for the determination of somatic (O) and flagellar (H) antigens using monovalent and polyvalent (O&H) *Salmonella* antiserum [19]. Also, *Shigella* serotypes was confirmed by slide agglutination test using *Shigella* antiserum (Difco Laboratories) according to the manufacturer’s instructions.

### 2.3 Enumeration of *Salmonella* and *Shigella* Isolates

The bacterial isolates were counted according to [20]. In brief, 10 ml or gm of each sample was aseptically introduced into 90 ml of sterile normal saline solution and homogenised by shaking followed by further decimal dilutions up to 10⁻⁵ concentrations. A 0.1 ml quantity of appropriately diluted sample was used to inoculate freshly prepared media by spread plate method, and then incubated at 37°C for 24 h. The present colonies were counted and recorded after incubation at 37°C for 24 h, to get the total bacterial count in CFU/ml or gm. The bacterial count was expressed as log¹⁰ values of colony-forming units per millilitre (CFU/ml or gm).

### 2.4 Molecular Determination of Virulence Genes

Uniplex PCR (uPCR) assay was used for the detection of virulence genes of *Salmonella* (*invA* gene) and *Shigella* (*invC, ipaH and virA* genes) isolates. Shortly, extraction of DNA was performed according to QIAamp DNA mini kit instructions. The cycling conditions and specific primers are illustrated in Table (1). The PCR products were electrophoresed on a 1 % agarose gel at 100 V. The agarose gel was stained with 0.5 μg/ml ethidium bromide. The DNA band was visualised by gel documentation system (Biorad, USA) [16,21,22,23].

### 2.5 Statistical Analysis

The data obtained were analysed using Statistics Package for Social Sciences (SPSS) software and Microsoft Excel 2007. This test combines ANOVA with comparison of differences between means of the treatments at the significance level of P < 0.05 [24].

### 3. RESULTS AND DISCUSSION

#### 3.1 The Prevalence of *Salmonella* and *Shigella* species in Milk and Dairy Products

Although milk and milk products have high nutritive value, they may contain different types of micro-organisms as a result of unhygienic conditions [25]. Bacterial contamination could generally occur from three main sources; within the udder, outside the udder and from the surface of equipment used for the milk handling and storage [26]. The consumption of raw milk and improper processed milk products remains a risk factor for foodborne illness particularly *Salmonella* and *Shigella* infection [7]. *Salmonella* and *Shigella* were shed in the feces of livestock such as cows and buffaloes and could contaminate milk during the milking process. Thus, a total of 24 (9.6 %) isolates from 250 samples (raw milk and dairy products) were identified as *Salmonella* 6.4 % (16/250) and *Shigella* 3.2 % (8/250) species as shown in Table (2 and 3). Among serotypes of *Salmonella* species (16/250): S. Typhimurium 37.5 % (6/16), S. Enteritidis, S. Tsevie 18.75 % (3/16) each, S. Infantis 12.5 % (2/16), S. Haifa and S. Virchow 6.25 % (1/16) each. Furthermore, the identified *Shigella* species (8/250) were S. dysenteriae 50 % (4/8), S. flexneri 25 % (2/8) and S. sonnei 25 % (2/8). A high prevalence of *Salmonella* was detected in kareish cheese (13.33 %), followed by raw milk (7.33 %) and ice cream (2.7 %), while *Shigella* was determined in high percentage in ice cream (8.1 %), and then raw milk (3.33 %) with its absence in kareish cheese and other examined dairy products.

The prevalence of *Salmonella* species in this investigation was consistent with [27, 28, 29] who detected *Salmonella* strains by 8.7 %, 7.7 % and 7.61 % from raw milk and milk products in Nigeria and India, respectively. Also, [30] as well as [31] investigated *Salmonella* species with the prevalence of 15 % (15/100) from milk and cheese and 2 % (16/800) from dairy products in...
Egypt, respectively. Additionally, [32] identified Salmonella species with a prevalence of 12 % (24/200) from milk and dairy products in Egypt, while [33] detected Salmonella species from raw milk in Ethiopia as 20 % (20/100). Other investigators had reported a wide range of prevalence of Salmonella (6.7 to 97.6 %) from bulk tank milk and milk filters in the United States [34]. Also, this finding detected a high occurrence of Salmonella species in Kareish cheese (13.33 %) in similarity to the previous studies in Egypt [35], while several investigators could not recover Salmonella species from Kareish cheese [36,37].

Furthermore, the current study revealed a lower prevalence of Shigella species. (3.2 %) that was compatible with [31] who isolated Shigella species from dairy products in a percentage of 1.4 % in Egypt. Whilst, previous studies [38, 39] showed a prevalence rate of Shigella species in raw milk (20 % and 17.5 % respectively), meanwhile, other investigators [40] showed a prevalence rate of Shigella species in 11.76 % ice cream. Overall, the results of this study on raw milk and dairy product samples indicated that inadequate hygienic and sanitation practices during milking, processing and manufacture [30,41].

Regarding to serotypes, S. Typhimurium was considered the major cause of Salmonella infection among the examined raw milk and dairy products which poses great public health hazards [42]. In this investigation, S. Typhimurium was the most dominant serotype amongst Salmonella isolates. Similarly, previous studies by [28,32] identified S. Typhimurium as the most predominant serotypes recovered from milk and cheese in Egypt and India. On the other hand, [30] detected S. enteritidis as the most common serotypes obtained from milk and cheese in Egypt. Moreover, this study showed the dominance of S. dysenteriae among Shigella species, while [43] found that S.flexneri was the most common species isolated from pedha (milk product) samples in India.

3.2 Total Viable Bacterial Count

As illustrated in Table (4), the total viable count of samples positive for Salmonella were ranged from 2.92±1.69 to 4.78±1.03 with an average of 4.47±0.97 log$^{10}$ CFU/ml or gm. Moreover, the total viable count for samples positive for Shigella was ranged from 3.99±0.9 to 4.47±1.09 with an average of 4.27±1.01 log$^{10}$ CFU/ml or gm. Fresh milk drawn from a healthy cow normally contains a low microbial load of less than 10$^{5}$ CFU/ml [44]. The presence of pathogenic bacteria such as Salmonella and Shigella species in the analysed samples is an indicator of poor hygiene and sanitation during milking and post milking processes [45]. The presence of Shigella in a very small amount (only 10 %) could cause disease (bacillary dysentery), which was easily transmitted and could cause big outbreaks [5]. Overall, the mean of total viable count of samples positive for Salmonella species (4.47±0.97 log$^{10}$ CFU/ml or gm) and samples positive for Shigella species (4.27±1.01 log$^{10}$ CFU/ml or gm) obtained in this study was similar to that obtained by [46] who detected Salmonella and Shigella count in raw cow’s milk with a range of 4.456 ± 0.443 log CFU/ml in India, respectively. Also, other investigators [38] noticed the Salmonella and Shigella count in raw milk with a mean 4.7 log CFU/ml and 6.04 log CFU/ml in Sudan, respectively. However, [20] found that the total Salmonella-Shigella count was ranged between 5.69-6.04 log CFU/ml in raw milk samples collected from dairy farms in Nigeria. Other researchers [40] detected the total Salmonella and Shigella count with a mean of 3.95 log CFU/ml and 3.7 log CFU/ml in ice cream, respectively. Previous study [47] reported that the mean of Salmonella count in local cheese was 6.3 log CFU/ml. This study showed that the quality of milk and dairy products resulted in the study areas were relatively poor. This was clear from the high values of total bacterial count (TBC) and there was the need for adequate sanitary measures and good personal hygiene at handling and different stages of production and consumption to reduce the public health risk.

3.3 Molecular Determination of Virulence Genes

A PCR assay targeting invA gene (target size: 284-bp) of Salmonella species as well as invC (target size: 875-bp), ipaH (target size: 600-bp) and virA (target size: 215-bp) genes of Shigella species was applied for identification of virulent strains of Salmonella and Shigella isolates. The invA gene was found in all Salmonella strains (16/16, 100 %) (Fig. 1). Furthermore, the ipaH and invC genes were detected in all Shigella strains (8/8, 100 %), while virA gene was absent in all Shigella strains (Fig. 2). A PCR targeting invasion (invA) gene conferred rapid identification of Salmonella isolates as all Salmonella serovars have the invA gene as a unique character [48]. The detection of invA gene...
by PCR was rapid, sensitive and specific method for the identification of Salmonella genus in many samples [10,11,49]. The invA is the first gene of an operon containing three or possibly more genes arranged in the same transcriptional unit [50]. This gene has been shown to be present and functional in most (if not all) Salmonella serotypes [51]. This study showed the presence of invA gene in 100 % of the Salmonella isolates tested. This result was consistent with other investigators [52] who detected invA gene in all isolated serovars of Salmonella from food samples. It was predictable since the invA is an invasion gene conserved among Salmonella serotypes, so all the Salmonella isolates were found highly invasive.

![Fig. 1. Representative agarose gel electrophoresis of Salmonella serovars showing PCR amplification for invA gene (284 bp). (L) ladder 100 bp; lane (1-10) positive samples, Neg (negative control), Pos (positive control).](image1)

<table>
<thead>
<tr>
<th>L</th>
<th>Pos</th>
<th>10</th>
<th>9</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>L</th>
<th>Neg</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 2. Representative agarose gel electrophoresis of Shigella serovars showing amplification for ipaH, invC and virA genes. (L) ladder 100 bp; lane (1-4): positive ipaH gene (600bp), Lanes (5-8): positive invC gene (875bp) and lane (9-12): negative virA gene (215bp). Neg (negative control), Pos (positive control).](image2)

<table>
<thead>
<tr>
<th>ipaH</th>
<th>invC</th>
<th>virA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Cycling of specific primers during uniplex PCR for virulence genes of *Salmonella* and *Shigella* species

<table>
<thead>
<tr>
<th>Target M.O.</th>
<th>Gene</th>
<th>Primer Sequence 5'-3'</th>
<th>Amplified product (bp)</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>No. of cycles</th>
<th>Final extension</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>invA</td>
<td>5'-GTGAAATTATCGCCACGTGCCGCA-3' 3'-TCATCGCAACGTCAAAGGAACC-5'</td>
<td>284</td>
<td>94°C/5 min.</td>
<td>94°C/30 sec.</td>
<td>55°C/30 sec.</td>
<td>72°C/30 sec.</td>
<td>35</td>
<td>72°C/7 min.</td>
<td>Oliveira et al., 2003</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>invC</td>
<td>5'-TGC CCA GTT TCT TCA TAC GC-3' 3'-GAA AGT AGC TCC CTA AAT GC-5'</td>
<td>875</td>
<td></td>
<td>60°C/45 sec.</td>
<td>72°C/45 sec.</td>
<td>72°C/10 min.</td>
<td></td>
<td></td>
<td>Ojha et al., 2013</td>
</tr>
<tr>
<td></td>
<td>ipaH</td>
<td>5'-GCCGTCAGCCACCT CGGAGACTAC-3' 3'- GTTCCCTTGACC GCCTTTCC GTAC GTG-5'</td>
<td>600</td>
<td></td>
<td>55°C/45 sec.</td>
<td>72°C/30 sec.</td>
<td></td>
<td></td>
<td></td>
<td>Jiménez et al., 2010</td>
</tr>
<tr>
<td></td>
<td>virA</td>
<td>5'-CGT CAT TCT GGC AAT CTC TTC ACA TC-3' 3'- GTA TGA GCT AAC TTC GTA AGC CCT CC-5'</td>
<td>215</td>
<td></td>
<td>60°C/30 sec.</td>
<td>72°C/30 sec.</td>
<td></td>
<td></td>
<td></td>
<td>Villalobo and Torres, 1998</td>
</tr>
</tbody>
</table>

Table 2. The prevalence of *Salmonella* species in milk and dairy products

<table>
<thead>
<tr>
<th>Types of samples</th>
<th>No. of samples</th>
<th><em>Salmonella</em> serovars</th>
<th>S. Typhimurium</th>
<th>S. Enteritidis</th>
<th>S. Infantis</th>
<th>S. Tsevie</th>
<th>S. Virchow</th>
<th>S. Haifa</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>150</td>
<td></td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>11(7.33%)</td>
</tr>
<tr>
<td>Ice cream</td>
<td>37</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2.7%)</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>30</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4(13.33%)</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>20</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Rice with milk</td>
<td>5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Cream</td>
<td>8</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td></td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>16(6.4%)</td>
</tr>
</tbody>
</table>
### Table 3. The prevalence of *Shigella* species in milk and dairy products

<table>
<thead>
<tr>
<th>Types Of Samples</th>
<th>No. of samples</th>
<th><em>S. dysenteriae</em></th>
<th><em>S. flexneri</em></th>
<th><em>S. sonnei</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>150</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5(3.33%)</td>
</tr>
<tr>
<td>Ice cream</td>
<td>37</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3(8.1%)</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Rice with milk</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Cream</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>250</strong></td>
<td><strong>4</strong></td>
<td><strong>2</strong></td>
<td><strong>2</strong></td>
<td><strong>8(3.2%)</strong></td>
</tr>
</tbody>
</table>

### Table 4. *Salmonella* and *Shigella* densities in raw milk and dairy products

<table>
<thead>
<tr>
<th>Positive samples</th>
<th>Salmonella serovars</th>
<th>log CFU/ml*</th>
<th>Positive samples</th>
<th>Shigella serovars</th>
<th>log CFU/ml*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. Typhimurium</td>
<td>4.66±1.06</td>
<td>1</td>
<td>S. dysenteriae</td>
<td>4.37±1.05</td>
</tr>
<tr>
<td>2</td>
<td>4.02±0.8</td>
<td>4.22±1.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.92±1.69</td>
<td>4.43±1.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.7±0.96</td>
<td>3.99±0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.46±0.92</td>
<td>4.1±0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.44±1</td>
<td>4.47±1.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S. Enteritidis</td>
<td>4.67±1.07</td>
<td>7</td>
<td>S. flexneri</td>
<td>4.29±1.04</td>
</tr>
<tr>
<td>8</td>
<td>4.12±0.83</td>
<td>4.26±0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4.78±1.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>S. Infantis</td>
<td>4.54±1.18</td>
<td>11</td>
<td></td>
<td>4.53±0.9</td>
</tr>
<tr>
<td>12</td>
<td>S. Tsevie</td>
<td>4.64±1.06</td>
<td>13</td>
<td></td>
<td>4.48±1.03</td>
</tr>
<tr>
<td>14</td>
<td>4.39±0.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>S. Haifa</td>
<td>4.51±0.99</td>
<td>16</td>
<td>S. Virchow</td>
<td>4.65±0.99</td>
</tr>
<tr>
<td>Over all mean</td>
<td>4.47±0.97</td>
<td>Over all mean</td>
<td>4.27±1.01</td>
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</tbody>
</table>

*Mean of log ± Standard deviation.
Moreover, three specific genes (invC, ipaH, and virA genes) to Shigella were detected by PCR. The invC gene was present among all of the Shigella species [16]. The ipaH gene is one of the most important virulence gene that is differentiated Shigella from Enteroinvasive E. coli (EIEC), since Shigella and EIEC have similar physio-biochemical characteristics [53]. The virA gene located upstream and transcribed divergently from icsA (virG), is involved in invasion and spreading, it is the only gene outside the main virulence gene operons to be regulated by the virB protein [54]. In this investigation, the invC and ipaH genes were detected in 100 % of Shigella strains tested, whereas virA gene was absent in all Shigella strains. This result was compatible to [55] who determined ipaH gene in all isolates of Shigella from food samples. [16] found invC gene in 96.7 % of Shigella strains isolated from human. In contrast, [21] determined virA gene in all Shigella strains isolated from mayonnaise.

4. CONCLUSION

Raw milk and dairy products could be a source of virulent strains of Salmonella species based on invA gene and Shigella species based on invC, ipaH, virA genes that has a public health hazard. Therefore, it should be overcome by proper hygienic measures during milking, handling of milk and manufacture of dairy products as well as effective training and education of the farmers to improve consciousness of milk borne zoonosis. This study improved the understanding of epidemiologic feature of salmonellosis and shigellosis and delivered a scientific basis for control and prevention of such diseases in Egypt.

ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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