Molecular differentiation and antimicrobial resistance patterns of *Salmonella enteritidis* and *Salmonella typhimurium* isolated from different food samples

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Abstract:

Our study was applied to differentiate between *S. enteritidis* (*Salmonella enteritidis*) and *S. typhimurium* (*Salmonella typhimurium*) because of their importance for public health, epidemiologists, and the poultry industry, and to assess their pathogenic potential and antimicrobial resistance. For this purpose a total of 135 different food samples was studied, where only 8 *Salmonella* isolates were detected. The presence of virulence and antimicrobial resistance genes associated with the isolated strains was investigated by PCR, and the results of the analysis of five virulence genes showed that 100% of the examined strains were positive for *invA*, *stn*, *fimH*, and *spvC* genes, while only 62.5% were positive for *pefA* gene. Regarding the antimicrobial resistance, the highest resistance frequencies of the isolates were to ampicillin followed by nalidixic acid. All ampicillin-resistant salmonella isolates harbored the *blaTEM* gene, while none of these isolates carried the *blaSHV* gene.

**Key words:** *Salmonella enteritidis; Salmonella typhimurium; RAPD PCR; virulence*

1- INTRODUCTION

Salmonella is one of the major crucial genera of pathogenic bacteria involved in foodborne illnesses, and it has many species distributed widely in different kinds of samples, including food animals, plants, pets, and even the surrounding environment (Abley, Wittum, Zerby, & Funk, 2012).

*S. typhimurium* and *S. enteritidis* are considered the most common causative agents of salmonella food poisoning all over the world (Gürakan, Aksoy, Ogel, & Oren, 2008). Currently, *S. enteritidis* remains the most common serovar isolated from African, European, Asian, and Latin American countries and the second most common serovar in North America and Oceania (Hendriksen et al., 2011).

Contamination of milk and dairy products with *Salmonella* species caused mainly by infected persons and contaminated environment, as contribution of natural udder infections to human food poisoning are rare (Mhone, Matope, & Saidi, 2012). It was known that poultry products are implicated as the most common sources of nontyphoidal Salmonellosis in humans (Dogru, Ayaz, & Gencay, 2010), but also raw milk and dairy products are becoming important sources of their infection (Oliver, Jayarao, & Almeida, 2005). *Salmonella* infection occurs mostly through the consumption of raw or contaminated food, the evaluated number of human food poisoning is greater than 93.8 million cases, with 155,000 deaths per year all over the world (Hendriksen et al., 2011).

Feng (1992) reported the ability of using many methods for detection of Salmonella, including the conventional culture method, immunological methods, and DNA-based
methods. Others found that, the application of PCR (Polymerase Chain Reaction) technique is a good choice for effective and rapid detection and identification of Salmonella (Van Lith & Aarts, 1994). RAPD (Random amplified polymorphic DNA) analysis is an attractive method for the detection and identification of microbes (Martinez-Murcia & Rodriguez-Valera, 1994; Stephan, 1996). It has the ability to detect polymorphism throughout the entire genome, and it does not require previous knowledge about the target DNA, so it allows one to start a blind search through the genomic DNA of an organism. Furthermore, RAPD is more proper techniques, because it uses short primers, a small amount of starting DNA (Gürakan et al., 2008). For these reasons, we choose it to identify, and differentiate between S. typhimurium and S. enteritidis as they give nearly the same disease symptoms, although RAPD is mainly used for molecular typing studies of bacteria.

The virulence of Salmonella depends on both of chromosomal and plasmid factors (Oliveira et al., 2003). Salmonella strains can carry different virulence genes needed for their invasion, replication, and survival in host cells. Most of these genes are located in groups called Salmonella Pathogenicity Islands, where they are often placed near to each other in the genome within the Salmonella chromosome (Mills, Bajaj, & Lee, 1995). There are many genes, which have been considered the main virulence genes responsible for Salmonellosis, including inv, spv, fimA, and stn genes (Sabbagh, Forest, Lepage, Leclerc, & Daigle, 2010).

Antimicrobial resistance as well as, appearance of Multiple drug resistance (MDR) in many serotypes of Salmonella is becoming an important health problem as they are responsible for increasing the mortality rates from foodborne illness (Hur, Kim, Park, Lee, & Lee, 2011; Rothrock et al., 2015). They have been occurring as a result to overuse or misuse of antibiotics (WHO, 2000). Controlling the antimicrobial resistance phenomenon may need scientific innovations through understanding the mechanisms involved in the evolution of antibiotic resistance in bacteria (Lynne, Rhodes-Clark, Bliven, Zhao, & Foley, 2008). Thus, we applied this study to report the incidence, pathogenic potentials, and antimicrobial resistance profile of S. enterica serovars Typhimurium and Enteritidis strains isolated from different food samples in Egypt using PCR.

2- MATERIALS AND METHODS

2.1. Sample collection
A total of 135 of different food samples, including 60 for raw milk, 40 for raw milk cheese (Kariesh cheese), and 35 for poultry eggs were collected from different localities in Sharkia governorate, Egypt. Samples were immediately cooled to 4°C and transported to the laboratory for further analysis.

2.2. Bacteriological examination
Isolation of Salmonella species. was carried out according to the ISO 6579-1 method (ISO-6579-1, 2017), then the suspected colonies were subcultured on XLD agar for 24 hours and pure colonies were subjected to Gram staining and biochemical and serological identification tests (Murray & Baron, 2007).

2.3. Molecular identification of Salmonella species and their virulence-associated genes
Extraction of bacterial DNA from samples was carried out using the QIAamp DNA Mini kit (QIAGEN GmbH, Hilden, Germany) with modifications from the manufacturer’s recommendations. Then the biochemically suspected *Salmonella* isolates were subjected to RAPD PCR for confirmation and differentiation between serotypes using short primer (5’-CGT GCA CGC-3’) with 9 bp in size (Gürakan et al., 2008). Isolates identified as *S. typhimurium*, and *S. enteritidis* were further subjected to molecular identification of *invA* (Oliveira et al., 2003), *spvC* (Huehn et al., 2010), *fimH* (Hojati, Zamanzad, Hashemzadeh, Molaie & Gholipour, 2013), *pefA* (Murugkar, Rahman, & Dutta, 2003), and *stn* (Murugkar et al., 2003) virulence associated genes.

A positive control of both *Salmonella* species was run alongside the tested isolates; this was kindly donated by the Biotechnology Unit, Animal Health Research Institute, Dokki, Giza, Egypt.

**2.4. Antimicrobial resistance pattern of *Salmonella* isolates**

The antimicrobial resistance pattern was determined by the disc diffusion technique following the protocol of CLSI (2011) (Clinical and Laboratory Standards Institute). The used antibiotics were AMP (ampicillin, 10 mg), C (chloramphenicol, 30 mg), NOR (norfloxacin, 10 mg), NA (nalidixic acid, 30 mg), T (tetracycline, 30 mg) and SMZ (sulfamethoxazole, 100 mg). Results were recorded and compared with the standard levels to know whether *salmonella* isolates were sensitive, intermediate or resistant. The interpretation of inhibition zones of test culture was determined according to (CLSI/ NCCLS, 2013). (Table 3)

**2.5. Molecular identification of antibiotic resistance genes**

Further molecular identification for *BlaTEM* (Colom et al., 2003), *BlaSHV* (Colom et al., 2003), *qnrA* (Robicsek, Strahilevitz, Sahm, Jacoby & Hooper, 2006), and *qnrS* (Robicsek et al., 2006) was applied as antibiotic resistance genes encoding for resistance to the antibiotics that give the highest resistance frequencies for the isolates.

**3- RESULTS AND DISCUSSION**

**3.1. Isolation and confirmation of *Salmonella* isolates**

*Salmonella* species have been considered as one of the most wide spread pathogens all over the world, and are responsible for different public health hazards (Scallan et al., 2011). The presence of *Salmonella* species in raw milk generally comes from feces of infected animals as the dairy cattle are considered the natural reservoirs of *Salmonella* species (Callaway et al., 2005). After analysis of samples by conventional culture methods for detection of *Salmonella* species, The results present in table (1) showed that 3.3% of raw milk samples were positive for *Salmonella* species. With further identification, it was revealed that only *S. enteritidis* could be isolated from these samples.

Omar, Al-Ashmawy, Ramadan, and El-Sherbiny (2018) found that both *S. enteritidis* and *S. typhimurium* could be isolated from raw milk samples with the percentages of 40% and 26%, respectively. Variable incidences of *Salmonella* were reported in many previous
literatures as determined by Tadesse and Dabassa (2012), Tesfaw et al. (2013), and Gwida and Al-Ashmawy (2014). While other previous studies failed to isolate Salmonella from raw milk samples (Ekici, Bozkurt, & Isleyici, 2004; Mhone, Matope, & Saidi, 2012; Zeinhom & Abdel-Latef, 2014).

Table 1
Distribution of *S. enteritidis* and *S. typhimurium* recovered from the examined sources

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of the examined samples</th>
<th>Positive for <em>S. enterica</em> species</th>
<th><em>S. enteritidis</em></th>
<th><em>S. typhimurium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>60</td>
<td>2(3.3)</td>
<td>2(3.3)</td>
<td>0</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>40</td>
<td>1(2.5)</td>
<td>0</td>
<td>1(2.5)</td>
</tr>
<tr>
<td>Poultry egg</td>
<td>35</td>
<td>5(14.3)</td>
<td>1(2.9)</td>
<td>4(11.4)</td>
</tr>
</tbody>
</table>

* values in parenthesis are the percentage of the number examined.

Home-made Kareish cheese is a common type of cheese consumed widely in Egypt. Their manufacture and handling techniques is still unhygienic as it does not require boiling of the milk (Tahoun et al., 2016). It can be contaminated by pathogenic bacteria through contaminated raw milk, food handlers, dust, utensils, and insects. In the present study, only *S. typhimurium* could be isolated from cheese samples with a percentage in 2.5% (Table 1), while while Omar, Al-Ashmawy, Ramadan, and El-Sherbiny (2018) found only *S. enteritidis* in 3% of the examined Kareish cheese samples. Our findings were lower than those obtained by El-Kosi, 2001 and Gwida and AL-Ashmawy, 2014. However, several authors could not recover *Salmonella* species from Kareish cheese (Bahout and Moustafa, 2001 and Fadel and Ismail, 2009).

The eggs and egg products provide a reliable source of nutrition; as well as the role they play in the other products manufacture, and so their safety is important (Howard, O’Bryan, Crandall, and Ricke, 2012). Eggs can be contaminated either by penetration of the bacterium through the eggshell from the contaminated feces after oviposition, or by direct contamination of the egg components from the infected reproductive organs before oviposition (Martelli and Davies, 2012). In our study, *Salmonella* species could be isolated from egg samples in the percentage of 14.3%, including *S. enteritidis* and *S. typhimurium*. These results are in agreement with those obtained by Tsai, and Hsiang (2005), and Das, Hari, Shalini, Ganeshkumar, and Karthikeyan (2012).

*S. enteritidis* and *S. typhimurium* are very closely related serotypes and both of them are pathogenic for human and animals; therefore, the focus of the study was on both of *S.typhimurium* and *S.enteritidis*, which are considered as important causes of food poisoning in humans.

Identification of serotype-specific DNA marker for *S. typhimurium*, and its differentiation from *S. enteritidis* is very important for both the food industry and human health (Gürakan et al., 2008). Many scientists try to find an easy and rapid method for this purpose and find that application of RAPD analysis appears to be very essential in population genetics, and molecular taxonomy studies as it has the ability to discover even slight differences in the genetic make-up of closely related organisms (Williams, Kubelik, Livak, Rafalski, and
Tingey, 1990). Gürakan et al. (2008) concluded that using RAPD analysis with the a serotype-specific marker for *S. typhimurium* in the primer (5′-CGT GCA CGC-3′) is of potential use as this primer appeared to produce a distinguishable pattern against this serotype. So in our study, we use this Primer with 9 bp in size (Figure 1) for testing 8 Salmonella strains which were identified as *S. typhimurium* and *S. enteritidis*. The results cleared that the amplification pattern of *S. typhimurium*, with the intenses 300 and 700-bp products, were found to be unique among the 5 *S. typhimurium* isolates, whereas the three isolates of *S. enteritidis* produced a 300-bp amplification band only with this primer under the same RAPD-PCR conditions (Figure 1). Therefore, a 300-bp band could not be specific for *S. typhimurium*, while 700-bp amplification band could be used as a specific DNA marker for *S. Typhimurium*.

![Fig. 1. Representative RADP-PCR products of *S. enteritidis* and *S. typhimurium* serotypes in 1.2% agarose gel: Lane L = 100-bp ladder plus marker; Lanes 1,2,3 = *S. enteritidis*; Lanes 4,5,6,7,8 = *S. typhimurium*; pos. = positive control; neg. = negative control.](image)

The specificity of the 700-bp band for *S. typhimurium* in our study was in accordance with the results of McClelland's homology comparative study (McClelland et al., 2001) because they had discovered the presence of nonhomologous regions on DNA among these bacteria, so it was thought that the polymorphic 700-bp region could be located in the nonhomologous region of *S. typhimurium*, which differentiate it from closely related *Salmonella* serotypes and other Enterobacteriaceae members.

Our results were similar to that of Gürakan et al. (2008), at which the same primer generated a unique and distinguishable amplification pattern with *S. typhimurium* at 700 bp, and 300 bp regions.

### 3.2. Molecular identification of virulence-associated genes

It is worth mentioning that there are some differences in the virulence of *Salmonella* species, although all its serotypes can be considered as potentially pathogenic (Karasova, Havlickova, Sisak, & Rychlik, 2009). The detection of multiple genetic profile in one strain may be caused by gaining or losing of genes for increasing its adaptation to the host (Prager et al. 2000). To assess the virulence potential of *Salmonella* isolates from the examined sources, the presence of *invA, stn, fimH, spvC, and pefA* genes virulence associated genes was determined in the *Salmonella* isolates as recorded in table (2).
Table 2: Distribution of virulence associated genes present in different serovars of Salmonella isolated from different food samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>+ve samples for Serotypes</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>invA</td>
</tr>
<tr>
<td>Raw milk</td>
<td>2 S. enteritidis</td>
<td>2(100%)</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>1 S. typhimurium</td>
<td>1(100%)</td>
</tr>
<tr>
<td>Poultry egg</td>
<td>1 S. enteritidis</td>
<td>1(100%)</td>
</tr>
<tr>
<td></td>
<td>4 S. typhimurium</td>
<td>4(100%)</td>
</tr>
</tbody>
</table>

The investigated genes comprised the Salmonella invasion gene invA gene which associated with Salmonella pathogenicity islands (SPIs), the adhesiveness related gene fimH, the gene spvC (Salmonella plasmid virulence gene C) from the spvC operon, stn gene involved in enterotoxin production and pefA gene (plasmid-encoded major fimbriae). These virulence genes have been distributed widely among isolates of different sources, including humans, animals, and the environment, but with some diversity (Hendriksen, 2010).

The occurrence value of invA gene cleared its presence in 100% of the examined Salmonella isolates (S.typhimurium and S. enteritidis). This detection rate is in agreement with previous reports (Oliveira et al., 2003; Amini et al., 2010) as these studies described invA gene as a marker for the Salmonella serotypes in molecular detection by PCR. The invA gene plays an important role in entry of bacteria into the host epithelial cells and supposed to be a component of the inner membrane of SPI-1 dependent type III secretion system (TTSS-1) virulence apparatus (Hur et al., 2011), and it was found that this gene present in high frequency only in serotypes that have the ability to cause severe hazards in humans (Ben-Barak et al., 2006; Borges et al., 2013).

In our work, we examined Salmonella isolates for the presence of stn gene. The results cleared the presence of this gene in all S.typhimurium and S. enteritidis isolates (Table 2). This was in agreement with other authors (Dinjus, Hanvel, Muller, Bauerfeind, and Helmuth, 1997; Soto, Rodriguez, Rodicio, Vila, and Mendoza, 2006). The stn gene may be considered as a potential cause for diarrhea caused by Salmonella, which is a complex phenomenon caused by several mechanisms, including production of enterotoxin, which is mediated by this gene (Chopra, Houston, Peterson, Prasad, and Mekalanos, 1987).

The high detection rate of inv and stn genes by PCR in the examined strains expressed their virulence in the host, and suggested the presence of these two genes among S. enterica isolates (De Oliveira et al., 2003; Murugkar et al., 2003).

Another investigated virulence gene in this study was a fimH gene, which was detected in all isolates produced 164 bp DNA fragment as it was cleared in the table (2), and our result was in accordance to that of Dhanani et al. (2015). This gene has been described as the main gene responsible Salmonella adhesion and invasion (Althouse, Patterson, Fedorka-Cray, and Isaacson, 2003; Guo, Lasaro, Sirard, Kraehenbuhl, and Schifferli, 2007) and recently was shown to have a crucial role in bacterial transcytosis through M- cells, which has a great role in activation of the mucosal immune response (Ohno & Hase, 2010).
The *spvC* gene is required for full expression of virulence in Salmonella (Haneda, Okada, Nakazawa, Kawakami, and Danbara, 2001) as it encourages the bacterial growth, and survival within the host (Chiu and Ou, 1996). It was detected in all *Salmonella* serotypes belonged to both *typhimurium* and *enteritidis* (Table 2). In accordance, Soto et al. (2006) reported the presence of *spvC* in all the isolates and this showed that these strains have the ability to cause the systemic infection as they have the plasmid-borne virulence characters. In contrast, others did not detect *spvC* gene in any *Salmonella* isolates from the examined samples of his work (Araque, 2009). Different frequencies for the virulence plasmid gene *spvC* were found by other authors (Amini et al., 2010; Derakhshandeh, Firouzi, and Khoshbakht, 2013).

*PefA* (700bp fragment) gene showed amplifications only in 5 *Salmonella* isolates (2 of them belonged to serovar enteritidis and 3 belonged to typhimurium)( Table 2). These results were similar to other comparable studies (Murugkar et al., 2003; Das et al., 2012) who found that only some of their *Salmonella* isolates were positive for *pefA* gene. The *pefA* gene can be serovar specific (Rotger and Casadesus, 1999) and is encoded by virulence plasmids (Gulig et al., 1993). In addition, this virulence gene may not present in all isolates of a plasmid-bearing serovar (Rotger and Casadesus, 1999), and this could explain the presence of this gene in a low proportion in the examined isolates shown in Figures (2).

![Agarose gel showing polymerase chain reaction amplification products](image)

**Fig. 2.** Agarose gel showing polymerase chain reaction amplification products of *invA* gene (284 bp) (a), *stn* gene (617 bp) (b), *fimH* gene (164 bp) (c), *spvC* gene (467 bp) (d), and *pefA* gene (700 bp) (e). lane 1, 100 bp ladder; lane neg., negative control; lane, pos., positive control of each investigated gene; lanes, 1-3, *S. enteritidis* isolates and lanes, 4-8 *S. typhimurium* isolates.

### 3.3 Antimicrobial resistance

- **Phenotypic antimicrobial resistance.**

Several researchers suggest that the extensive or unplanned use of antimicrobials can lead to resistance of several types of bacteria that, cause consumers' health hazards as they can reach them through products of animal origin (Ribeiro, Lincopan, Landgraf, Franco, and Destro, 2011). The appearance of new species of multidrug-resistant Salmonella have a great
effect on human health either through interference with their treatment or through the effect on other pathogens by dissemination of resistance elements to them (Frye and Jackson, 2013).

The antimicrobial resistance of the strains analyzed in the present study was tested against some classes of drugs recommended by the CLSI to treat Salmonella infections (Stevenson et al., 2007 and Tamang et al., 2011). Our results revealed that the highest resistance frequencies of the isolates were to Ampicillin, where 80% of S. typhimurium and 100% of S. enteritidis were resistant to it, followed by Nalidixic acid as 80% of S. typhimurium and 66.7% of S. enteritidis were resistant to it, and only 40% of S. typhimurium were resistant to Tetracycline. All S. typhimurium isolates listed in Table (3) were susceptible to Norfloxacin, while 80% were sensitive to Sulfamethoxazole and Chloramphenicol. Regarding S. enteritidis, all isolates were sensitive to Norfloxacin and Sulfamethoxazole, while only 66.7% strains were sensitive to Chloramphenicol. These results were in accordance with Chen et al. (2004) as he found that the examined strains gave the greatest resistance to Ampicillin, while Lamas et al. (2016) found resistance to Ampicillin in only 16.41%. In contrast, Campioni, Bergamini, and Falcao (2012) cleared that all the examined strains were susceptible to the Ampicillin.

In other studies, Lamas et al. (2016) found that the highest level of resistance was to Sulfamethoxazole followed by Nalidixic acid and Ampicillin. On the other hand, the antimicrobial resistance profile showed that 21.5%, 41.5% and 28.1% of the examined strains were resistant to Nalidixic acid, respectively (Kottwitz et al., 2011; and Campioni et al., 2012).

Table 3: Breakpoint values of each antimicrobial agent and phenotypic antimicrobial susceptibility profiles of the tested isolates used in this study

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Disc Potency μg/disc</th>
<th>Diameter of inhibition zone (mm)</th>
<th>S. typhimurium isolates (n=5)</th>
<th>S. enteritidis isolates (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R (%)</td>
<td>I (%)</td>
</tr>
<tr>
<td>AMP</td>
<td>10 μg</td>
<td>≤13</td>
<td>14-16</td>
<td>≥17</td>
</tr>
<tr>
<td>C</td>
<td>30 μg</td>
<td>≤12</td>
<td>13-17</td>
<td>≥18</td>
</tr>
<tr>
<td>Nor</td>
<td>10 μg</td>
<td>12</td>
<td>13-16</td>
<td>17</td>
</tr>
<tr>
<td>SMZ</td>
<td>30 μg</td>
<td>13</td>
<td>14-18</td>
<td>19</td>
</tr>
<tr>
<td>NA</td>
<td>30 μg</td>
<td>≤14</td>
<td>15-18</td>
<td>≥19</td>
</tr>
<tr>
<td>T</td>
<td>100 μg</td>
<td>≤10</td>
<td>11-15</td>
<td>≥16</td>
</tr>
</tbody>
</table>


Many studies have shown that S. enteritidis does not develop resistance as S. typhimurium, which possesses the highest number of antimicrobial resistances (Capita, Alonso-Calleja, & Prieto, 2007; Alvarez-Fernandez, Alonso-Calleja, García-Fernández, and Capita, 2012). While our results showed that S. typhimurium possesses resistance for Ampicillin, Nalidixic acid, and Tetracycline with the percentage of 80%, 80% and 40% for
each, respectively, while *S. enteritidis* developed resistance for Ampicillin, Nalidixic acid with the percentage of 100% and 66.7% for each, respectively.

3.4. Detection of antimicrobial resistance-associated genes

It has been reported that there are four classes A, B, C, and D of β-lactamases which confer resistance to β-lactam antibiotics. TEM and SHV enzymes, which are belonged to class A β-lactamases are generally found in Gram-negative bacteria (Bradford, 2001). TEM-1 family β-lactamase gene was most often associated with strains resistance to Ampicillin (Chen et al., 2004). The distribution of resistance genes among phenotypic Ampicillin resistant *Salmonella* serovars isolated from different food products in our study is shown in figure (3). Where All these isolates harbored the *blaTEM* gene, while none of these isolates carried the *blaSHV* gene (Figure 3). Rayamajhi et al. (2017) gave similar results as they revealed that the *blaTEM* gene was amplified in all Ampicillin-resistant isolates, while SHV β-lactamases was not detected in these Ampicillin-resistant *Salmonella* isolates. These findings are in agreement with previously published data, which describe the presence of correlation between the phenotypic resistance to Ampicillin and the production of TEM lactamase enzymes (Hur et al., 2011; Zou, Keelara, and Thakur, 2012). The wide use of Ampicillin has led to the spread of salmonella resistance and consequently the appearance of β-lactamases in pathogens (Sow, Wane, Diallo, Boye, and Aidara-Kane, 2007).

![Fig. 3. Agarose gel showing polymerase chain reaction amplification products of *blaTEM* gene (516 bp) (f), *blaSHV* gene (392 bp) (g), *qnrA* gene (516 bp) (h), and *qnrS* gene (417 bp) (i).](image)

Nalidixic acid has been known as the most common used antimicrobial for both human and veterinary treatments (Tamang *et al.*, 2011). Recently, plasmid-mediated quinolone resistance (PMQR) has emerged in many bacterial species all over the world, including Salmonella and other Enterobactericeae (Robicsek, Jacoby, and Hooper, 2006). The *qnrA* gene was identified as the first PMQR determinant (Martinez-Martinez, Pascual, and Jacoby, 1998). Subsequently, other *qnr* alleles have been described, as *qnrB*, *qnrS*, *qnrC*, and
The qnrD (Cavaco, Hasman, Xia, and Aarestrup, 2009). The qnrA and qnrS genes were the only PMQR determinants were tested in our work. Our results for this part indicated that all PCR screenings performed for qnrA and qnrS were negative in all examined isolates as shown in figure (3). These results are in contrast to results of Gunell et al. (2009) who indicated that the plasmids containing qnr genes are spreading among isolates from different locations, times, and serovars. The Salmonella strains showed phenotypical resistance to nalidixic acid without harboring the qnrA and qnrS genes, indicating that these strains possess another resistance mechanism to this antibiotic needing further research (El-Sharkawy et al., 2017).

On the other hand, strains that were phenotypically sensitive to Chloramphenicol, Norfloxacin, and Sulfamethoxazole may also carry resistant genes to these antibiotics, but these genes may be silent in vitro in these Salmonella serovars, or may not be expressed at the time of the analysis, however, these silent genes can be activated invivo or, spread to other bacteria especially under antimicrobial pressure as in previous reports (Maynard et al., 2003; Ma, Wang, Yu, Zhang, and Liu, 2007). Peirano et al. (2006) and Ribeiro et al. (2011) also reported that it was not always possible to correlate phenotype and genotype in the isolates showing resistance. In the future, it is recommended to design intervention strategies to reduce the progression of antimicrobial resistant bacteria through understanding of the mechanisms by which emerges and spreads, as these resistant bacteria can be transferred to humans through the consumed food (Suez et al., 2013). Minimize the use of antibiotics in food animals, is also essential to control the rapid spread of these bacteria.

4- CONCLUSION

It was concluded that contamination of the examined samples currently accounts for a small percentage of Salmonella foodborne microbes, in spite of that, it is clear that the consumption of products made with raw milk, and poultry egg presents some risk. On the other side, the PCR detection of invA, spvC, pefA, FimH and stn genes among the isolated Salmonella strains also indicated the higher risk of foodborne infections caused by these isolates in humans. Currently, the foremost challenges are discovering how Salmonella acquires virulence factors, as it is known that Salmonella species have the ability to lose and acquire new virulence factors over time to adapt to new hosts. This needs further research as these information allows a better approach in the study, and reduction of Salmonella virulence.

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التمييز الجزيئي لسالمونيلا انتريتيديس وسالمونيلا تيفيموريوم المعزولين من الأطعمة المختلفة وأنماط مقاومتهما

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تم تجميع 135 عينة من الأطعمة المختلفة بواقع 60 عينة من اللبن الخام، 40 عينة من الجبن القريش، 35 عينة من البيض. أوضحنت النتائج أن 8 عينات كانت إيجابية لمعضروب السالمونيلا بواقع 3 عينات خاصة بعترة السالمونيلا انتريتيديس و5 عينات خاصة بعترة السالمونيلا تيفيموريوم. تم استخدام تقنية تفاعل البلمرة المتسلسل للكشف عن جينات الضراوة وكذلك المقاطعة للمضادات الحيوية.

أظهرت النتائج تواجد أربعة جينات خاصة بالضراوة، من العترات بنسبة 100% من العترات *invA, stn, fimH, spvC* (جينين *invA, stn*). أوضحنت النتائج أن العترات المعزولة بنسبة 62% خاص بهن واحد فقط من جينات الضراوة (جين *pefA*). بعد أن كان هذه العترات المعزولة كانت أعلى مقاومة للأمبيسلين، ثم حامض الناليديكسيك. جميع عترات السالمونيلا المعزولة لأنابيب السالمونيلا كانت تحمل جين *blaSHV*، في حين أن أيا من هذه العترات كان يحمل جين *blaTEM*. 183