# Isolation and Molecular Detection of *Salmonella Infantis* from Milk and Children with Gastroenteritis in Babylon Province, Iraq

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## Abstract

Two hundred and ten samples were included in this study. The samples encompassing of 90 cattle milk samples obtained from local markets, and 120 stool samples obtained from children with gastroenteritis of age less than 12 years from both genders who were attending to the Babylon maternity and children's hospital. The samples were collected during the interval from February to August 2018 in Babylon province.

All these 210 samples were subjected to many tests for isolation and identification of *Salmonella* species by using bacterial culturing, biochemical tests, and API20E test system. The results showed that the prevalence of identifying *Salmonella* isolates were 69 (32.9%) out of 210 samples, distributed as (25/90) (27.8%) market milk and (44/120) (36.7%) stool samples. The identified *Salmonella* isolates were subjected to serotyping by monovalent antisera. It was found that 4 out of 25 isolates (16%) in market milk samples and 16 out of 44 isolates (36.4%) in stool samples that belong to *Salmonella infantis*. PCR technique was achieved to identify *invA* and *invF* virulence genes in *Salmonella infantis* strains. All 20 isolates of *Salmonella infantis* in market milk and stool samples were examined. The results revealed that, *invA* gene was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, while the *invF* gene was present in 2 strains of the market milk *Salmonella infantis*, but existing in all strains of the stool *Salmonella infantis*.

***Keywords:*** *Salmonella infantis, invA gene, invF gene, gastroenteritis, Milk, Gastroenteritis, Antibiotic Resistance.*

## Introduction

*Salmonella* is one of the most essential zoonotic bacterial pathogen affects humans, which causes foodborne infection all over the world with serious implications in developing countries [1], and it is a major foodborne problem with frame animals such as cattle [2]. The milk is a potential food for the transmission of diverse foodborne bacteria to humans. In addition to the poultry foodstuffs are the main sources of human nontyphoidal salmonellosis. Additionally the raw milk and dairy products are considered as the other causes [3]. The raw milk is contaminated with bacteria from several sources like feces of infected animals, infected udder, skin, dust, feed and milking equipment [4].

*Salmonella* species are the etiological agents of acute gastroenteritis. In spite of the global human health, *Salmonella* infections impact has not been estimated where gastroenteritis is represented the main cause of morbidity and mortality [5,6]. *Salmonella* is transmitted to humans by consumption of contaminated animal foodstuffs including eggs, milk, poultry, pork, beef, and seafood [7], contaminated water, contact with infected animals, or with an infected person who suffering from diarrhea. *Salmonella* is divided into more than 2500 serovars based on its H antigens [8]. These serovars are found in animal origin food products like *Salmonella enteriditis*, *Salmonella gallinarum*, *Salmonella typhimurium*, *Salmonella infantis* and *Salmonella weltevreden* [9].

*Salmonella Infantis* is the most recurrence strain in various countries, involving Asian countries, it was detected and isolate from animals, humans and vegetables [10].

The pathogenicity of *Salmonella* is frequently dictated by various virulence genes. The products of these genes are effector proteins and comprise *sipA*, *sipB*, *sipC*, *invA*, *invF, sifA, hilA*, *hilC* and *hilD* [11]**.** Traditional *Salmonella* isolation methods are based on growth in a culture selective medium and serotyping which is considered as the standard methods for *Salmonella* confirmation, but it is time consuming. Thus, a quick technique is needed for the identification of *Salmonella* strains like using PCR assay for rapid identification and confirmation [12].

There are no previous studies dealing with the occurrence of *Salmonella infantis* conducted in Babylon province. This study, therefore, aims to estimate the prevalence and detect *invA* and *invF* virulence genes in *Salmonella infantis* isolates obtained from market milk and stool samples of children with gastroenteritis in Babylon province, Iraq.

## Materials And Methods

### Sample Collection and Processing

The market milk and stool samples that included in this study were collected during the interval from February to August 2018 in Babylon province.

### Milk samples collection

Total of 210 samples, including 90 raw milk (market milk), were randomly collected from different local markets**.** The samples were placed in sterile tubes containing Selenite broth, maintained in an ice box, then transferred to the laboratory on the same day within two hours of collection for inoculation on selective medium.

### Stool sample collection

A total of 210 samples that composed of 120 stool samples were collected from children suffering from gastroenteritis. Samples were obtained from patients did not receive any antibiotics of age less than 12 years from both genders who were attending to the Babylon maternity and children's hospital. One gram of stool sample from each patient was placed in sterile tube containing 5 ml of Selenite broth, and maintained in an ice box, then transferred to the laboratory on the same day within two hours of collection for inoculation on selective medium.

### Laboratory Diagnosis

### Bacterial isolation, identification and serotyping

All (raw milk and stool) samples were inoculated onto the surface of MacConky agar, and incubated at $37^0$C for 24-48 hour, then sub-cultured onto the surface of selective media, including Eosin Metheline Blue, S.S and XLD agar, and then incubated at $37^0$C for 24 hours [13].

The identification of colonies was depended on the morphological properties (colony size, shape, color, borders and texture). Bacterial colonies displaying the distinctive morphology were probably identified as *Salmonella* by Gram's stain. The colonies of pathogens suspected to be *Salmonella* were subjected to biochemical tests such as (Catalase, Oxidase, Indole, Methyl red, Vogas-Proskaur, Citrate utilization, Urase, Gelatin hydrolysis, and Lactose fermentation tests) [14]. The analytical profile index (Api20E) system was used to confirm diagnosis of the *Salmonella* isolates by biochemical tests [15]. To determine *Salmonella infantis* strains the identified *Salmonella* strains were then tested for serotyping diagnosis based on the Kauffman-White Scheme with polyvalent and monovalent O and H antigens [16].

### Molecular detection of virulence genes in *Salmonella infantis*

The detection of virulence genes (*invA* and *invF*) in *S.infantis* was done as described by other workers [17-18]. Detailed sequences of the primers are listed in Table (1).

**Table 1: Oligonucleotide Primers sequence and amplicon size**

| Target gene | Primer sequence | Product size (bp) |
|---|---|---|
| invA | Forward:<br>5′-GTGAAATTATCGCCACGTTCGGGCAA-3′<br>Reverse:<br>5′-TCATCGCACCGTCAAAGGAAC-3′ | 284 |
| invF | Forward:<br>5′AAGGGATCCATGTCATTTTCTGAAAGCGACAC-3′<br>Reverse:<br>5′-GTTGTAGGGAAAGCTTCTCCAGTAATG-3′ | 918 |

Bacterial cells were suspended in 1 m1 of sterile D.W and subjected to a dry bath at 95°C for 10 min. then was cooled directly on ice and and used as DNA template for PCR. A single reaction mixture was completed in a final volume of 10µl containing 5µl master mix, 0.5µM of each primer and D.W water to 10µl final volume. The thermal cycler conditions were carried out at 95°C for 30 seconds as pre-denaturation, 35 cycles consisting of denaturation at 95°C for one second, primer annealing at 58°C for 5 seconds and extension at 72°C for 15 seconds. Final extension at 72°C for 1 minute. PCR amplicon was ran on 1.5 % agarose gel, stained with ethidium bromide then visualized on UV transilluminator and photographed.

## Results

Two hundred and ten samples were included in this study. Samples were covering 90 cattle milk samples obtained from local markets, and 120 stool samples obtained from children with gastroenteritis. Samples were processed for isolation and identification of *Salmonella* by using bacterial culturing, biochemical tests, and API20E test system. The results showed that the prevalence of identifying *Salmonella* isolates were 69 (32.9%) out of 210 samples, distributed as 25 (27.8%) among 90 market milk samples, and 44 (36.7%) among 120 stool samples. The frequency of presumptive *Salmonella* strains was isolated from 210 samples, as shown in the Table (2).

**Table 2: Distribution of *Salmonella* isolates according to the type of samples**

| Type of samples | No. of samples | No. of positive samples | Rate |
|---|---|---|---|
| Market milk samples | 90 | 25 | 27.8 % |
| Stool samples | 120 | 44 | 36.7 % |
| Total | 210 | 69 | 32.9 % |

The all identified *Salmonella* (69) isolates were subjected to serotyping by monovalent antisera. It was found that *Salmonella infantis* was detected in 20 (29%) samples distributed as 4 (16%) out of 25 market milk samples, and 16 (36.4%) out of 44 stool samples (Table 3).

**Table 3: Distribution of *Salmonella infants according* to the type of samples**

| Type of samples | No. of samples | No. of positive samples | Rate |
|---|---|---|---|
| Market milk samples | 25 | 4 | 16 % |
| Stool samples | 44 | 16 | 36.4 % |
| Total | 69 | 20 | 29 % |

**DNA was extracted from all (20) *Salmonella infantis* isolates and used as a template to identify the *invA*, *invF* virulence genes by using the PCR assay. The results found that, *invA* gene (284 bp) was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, the results are shown in figure (1). The *invF* gene (918 bp) was present in 2 strains of the market milk *Salmonella infantis* isolates, but existing in all strains of the stool *Salmonella infantis* isolates, the results are shown in figure (2).**

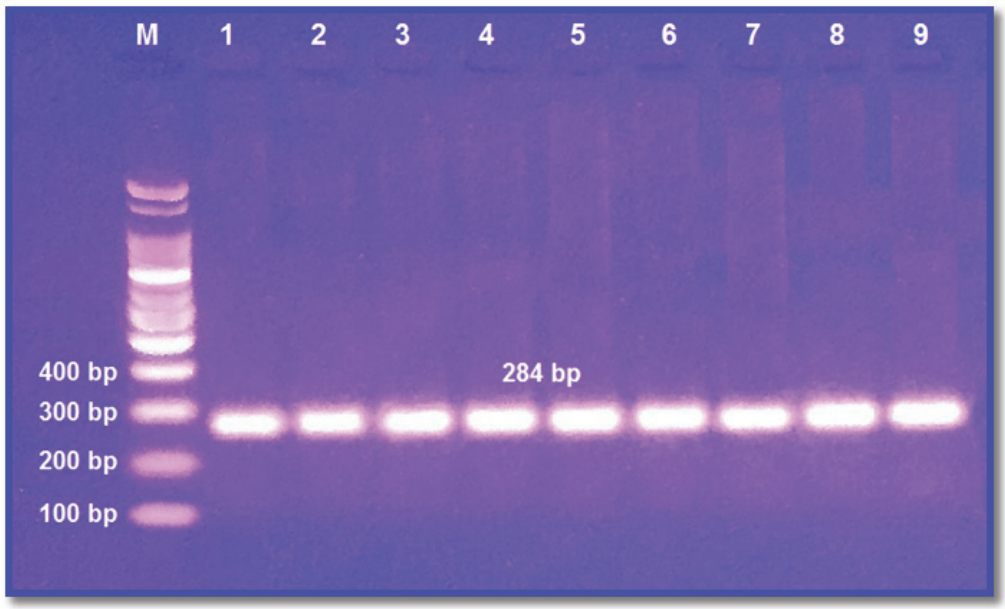


**Figure 1: Agarose gel electrophoresis of *Salmonella infantis invA* gene detection. Lane M: marker with 100 bp ladder, lane 1 to 9: positive stool and market milk isolates, *invA* gene amplicon size: 284 bp.**

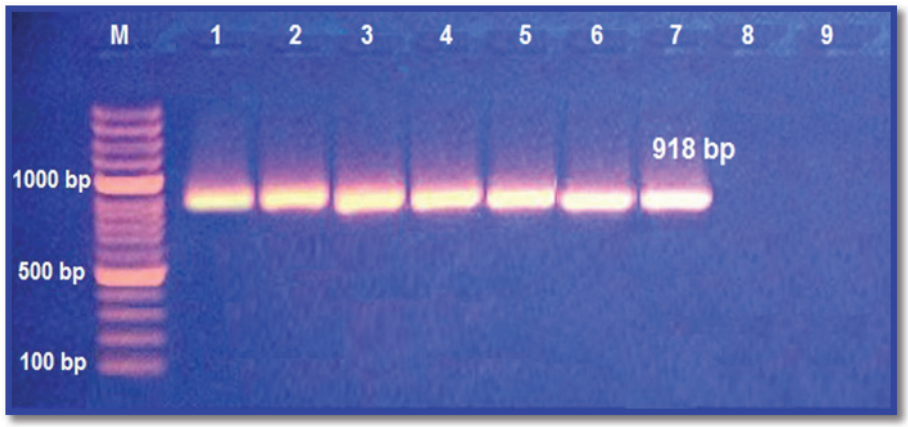


**Figure 2: *Salmonella infantis invF* gene amplicon. Lane M: marker with 100 bp ladder, lane 1 to 5: positive stool isolates, lane 6 and 7: positive market milk isolates, while lane 8 and 9: negative market milk isolates, *invF* gene amplicon size: 918 bp.**

## Discussion

This study provided an estimation of the prevalence and detect *invA* and *invF* virulence genes in *Salmonella infantis* isolates collected from market milk and stool samples of children with gastroenteritis in Babylon province, Iraq.

In the present study, the findings demonstrated that the prevalence of identifying *Salmonella* by using bacterial culturing, biochemical tests, and API20E test system in market milk samples was found to be (27.8%). This may be associated with the fecal contamination of raw milk by food borne bacteria in the course of its gathering or lower storage temperature of milk, loss of electricity in addition to post processing contamination, that offer proper conditions for variety of pathogenic bacteria to grow such as *Salmonella*.

This result corresponded to the result recorded in Southwest Ethiopia by [19], who found that the incidence of *Salmonella* spp. in raw milk was (20%). Different other previous study recorded in Egypt was carried out in 2014, and the results revealed that (12%) of the milk samples were positive for *Salmonella* species by using the conventional culture methods [20]. On the other hand, the result of the present study is in contrast with the results of the previous studies obtained from [21,22], *who indicated that Salmonella* species did not isolate and detect in examining raw milk samples.

In addition, the results showed the prevalence of *Salmonella* species in stool samples was (36.7%). The obtained result is lower than the result of the other study in Al-Muthanna Province, Iraq [23], who found that the prevalence of *Salmonella* species is (2.9%) in stool samples among children who their age under 9 years old. Another study in Al-Diwaniya, Iraq, recorded that *Salmonella* spp. isolated from stool samples of children under 3 years of age at a rate of (7.9%) were detected by culture and biochemical tests [24]. Variable prevalence of *Salmonella* was reported in many previous researches from Al-Diwaniya governorate, Iraq, there were (14.47%, 8.47%, and 10%) respectively [25].

The occurrence of the same virulence genes (*invA* and *invF*) in raw milk and human stool samples demonstrates that the distribution and dissemination of virulence genes, although the *S. infantis* source of infection not known due to lake of background information, but the milk are one of the most common sources known of human salmonellosis.

The *invA* gene of the *Salmonella* spp., triggers the attack of the host and initiate infection, and subsequently increasing the degree of pathogenicity of the bacterial strains [26]. The *invA* gene is considered a universal genetic marker for the *Salmonella* identification since it exists in almost all *Salmonella* serovars [27]. Moreover, *invF* is expressed as a response to environmental signals. The exsitance of these genes is important in the *Salmonella* clinical significance [28].

## Conclusion

*invA* gene was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, while the *invF* gene was present in 2 strains of the market milk *Salmonella infantis*, but existing in all strains of the stool *Salmonella infantis*.

### Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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