
Study on the Incidence of Salmonella Species in Different Food Samples by Using Cultural and Rapid *invA* Gene Specific PCR-Based Assay

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Abstract: *Salmonella* is the most common zoonotic pathogen around the world, there is an inadequate capacity of tests to detect this pathogenic bacterium in Libya. Therefore, this study was conducted to investigate the presence *Salmonella* spp in various foods from different food establishments in Tripoli, Libya. A total number of 370 samples were taken from 35 confectionery premises (170 samples of cakes, 25 of tarts), 11 poultry butcheries (55 samples of chicken meat), and 2 cattle butcheries (120 samples of camel meat). The isolates of *Salmonella* bacteria were investigated and identified by conventional cultures and biochemical methods such as (API20E). The typical *Salmonella* identified isolates were subjected to the PCR to detect *invA* gene. The results showed that 30/370 (8.11%) *Salmonella* spp were identified and distributed in 10 cake samples (5.9%), 2 tart samples (8%), 16 chicken meat samples (29.1%) and 2 camel meat samples (1.7%). The *invA* gene was detected in 22 isolates (73.33%), all *Salmonella* spp isolated from cakes and cattle meat samples are invasive strains. Overall, *Salmonella* spp is more abundant in poultry butchers than other food establishments in Tripoli, Libya, inclusion of PCR methods to detect *Salmonella* spp is highly recommended.

Keywords: *Salmonella*, *invA* Gene, PCR, Tripoli, Libya

1. Introduction

Salmonella is Gram negative, rod shaped bacterium which is one of the leading causes of human gastroenteritis around the world [19]. The salmonellosis is the name of the food-borne disease caused by *Salmonella* species [24]. There are several routes of this disease transmission, most of them are through consumption of contaminated food. Food sources of *Salmonella* included mainly egg, poultry, pork, beef, milk products, vegetables and fruits. [3, 2].

The contamination of the food by *Salmonella* is a major concern for public health [21]. The capability of *Salmonella* spp to develop gastrointestinal diseases is primarily associated with harbouring virulence genes, and

susceptibility of the host. The virulence genes are mainly located on the chromosome and the plasmids, the chromosomal invasion virulence gene *invA* is important to invade and survive in macrophages [7]. Several reports around the world have recruited this gene as target of Polymerase chain reaction (PCR) methods to detect *Salmonella* spp in clinical and non-clinical samples [19 18, 13, 11]. Therefore, this gene has become important diagnostic tool that can inform on the possible risk of *Salmonella* spp posed to public or animal health [5].

In Libya, the diagnosis of salmonellosis in most suspected cases is based on clinical features, not relying on specific laboratory methods. Therefore, neither regular monitoring of *Salmonella* infections, nor general databases have been established. Nevertheless, episodes of food poisoning caused

by unknown agents have been recently reported in different places in Libya. The growing levels of insecurity, weak health system in the country have seriously reflected on the capability to use advanced approaches in detection of outbreaks caused by microbial pathogens. Here, it is the first report determining the prevalence of *Salmonella* spp along with detection of *invA* gene of *Salmonella* from different food establishments in Tripoli, capital city of Libya. The main objectives were to (i) evaluate the prevalence of *Salmonella* spp in different sources of food and (ii) to determine the invasive strains of isolated *Salmonella*.

2. Materials and Methods

2.1. Ethical Approval

Fresh samples of food were used in this study; therefore, the ethical approval was not required.

2.2. Collection of Samples

Samples were collected from various foods taken from different establishments included 35 of confectionery premises, 11 of chicken butcheries and 2 of cattle butcheries located within Tripoli city in Libya. The total number of samples is 370 including 170 of cakes, 25 of tarts which were displayed for sale, 55 of chicken meat were collected from chicken butcheries and 120 of camel meat were collected from cattle butcheries. All of these samples were transported to microbiology laboratory at Biotechnology Research Centre in Tripoli city, Libya.

2.3. Isolation and Identification of *Salmonella*

2.3.1. Preparation of Samples

The samples were handled and processed under sterile conditions as described previously by (23) Twenty-five grams of each sample were added to 225 ml of pre-enrichment medium (buffered peptone water, CM509; Oxoid Ltd.). The mixture was homogenized for 1 to 2 min, and then incubated at 37°C±0.5 for 18 to 24 h. Following inoculation, 0.1 ml of each homogenized mixture was added into selective-enrichment media; 10 ml of Selenite broth, CM395 (Oxoid Ltd.) and incubated overnight at 37°C. Then, the loopful of broth streaked onto selective media; *Salmonella Shigella* Agar (SSA) (Oxoid Ltd.) and incubated at 37°C 18 to 24 h.

2.3.2. Biochemical Assays

Suspected colonies were firstly examined morphologically for shape and Gram staining. Next, the chemical activity of indole, methyl red, Voges-Proskauer, citrate utilization, triple sugar iron, and urease were confirmed. The pure cultures were made, and all isolates were further investigated by Analytical Profile Index) API 20E) (biomerieux Ltd.).

2.3.3. Motility Test

The motility medium agar was inoculated with *Salmonella* into the centre and incubated overnight at 37°C. The

diameters of motility zones were measured and interpreted in comparison with *S. enteritidis*. and *E. coli* K12, positive and negative control respectively.

2.4. Molecular Detection of *invA* Gene

2.4.1. Genomic DNA Extraction

Genomic DNA was prepared by using of GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, UK) kit. The purity and concentration of the extracted DNA was measured by using nanodrop 2000 (Thermo Scientific, UK).

2.4.2. PCR Amplification of *InvA* Gene

The primers used for detection of *invA* gene were same as described by [16], and are shown in table 1. PCR master mix was composed of 10 ng chromosomal DNA, 20 pmol forward and reverse primer (Sigma-Aldrich, UK), 1× PCR buffer (Promega, UK), 1.5 mM MgCl₂, 0.8 mM deoxynucleotide triphosphates (dntps), and 1.25 U Taq polymerase (Promega, UK). The PCR conditions were initial denaturation at 95°C for 1 min; 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 30s, and extension 72°C for 30s, followed by a final extension step of 72°C for 5 min. The positive control was *S. enteritidis* whereas *E. coli* K12 was negative control.

2.4.3. Agarose Gel Electrophoresis

The PCR products were separated and analysed on 2% agarose gel (Seakem LE Agarose, Cambrex) prepared in 1x TAE buffer (40mM Tris acetate, 1mM EDTA, pH8.2). 1 kb DNA ladder was used as a marker. The gel was run in TAE buffer at constant voltage of 100 v for 40 minutes. Then, it stained with ethidium bromide and visualised with ultraviolet light on a transilluminator gel documentation system (Syngene).

3. Results

3.1. Identification of *Salmonella* spp

The macroscopic examination was firstly performed on SS agar cultures, the transparent or translucent colorless colonies (with or without black center) were chosen for further biochemical examinations. The suspected colonies have been scored to be *Salmonella* if they showed positive reaction to triple sugar iron test and negative to urease test. The API20E kit was used for further confirmation.

The results showed that Out of 370 samples from different food sources, 30 (8.11%) isolates of *Salmonella* species were detected. All the 30 isolates were TSI positive, urease negative, ONPG negative, indole negative, methyl red positive, Voges Proskauer negative, and Citrate test positive, respectively.

Table 2 represent the results. majority of *Salmonella* spp were isolated from chicken meat (16/55, 29.1%), the samples originated from poultry are more contaminated by *Salmonella* spp than other sources. This was followed by cakes (10/170, 5.9%) and tarts (2/8, 8%), then and cattle (2/120, 1.7%).

3.2. Screening of Motile Salmonella

Salmonella isolates were examined for motility in order to explore the existence of flagella. The results revealed that all 30 isolates were able to spread actively across the agar, which indicate to presence of flagella. However, diameter zones of motility are relatively comparable with positive control *S. enteritidis* with no statistical differences observed.

3.3. Molecular Detection of *InvA* Gene

The isolates of *salmonella* spp were further investigated by PCR to detect *invA* gene. DNA was extracted from all isolates, and PCR was carried out by using *invA* specific primers. The results are shown in figure 1, which displays 1% agarose gel electrophoresis of positive *Salmonella invA* genes at size of 284 bp. The *invA* gene was observed in 22 strains (73.33%). All *Salmonella* isolates (100%) isolated from cattle and tarts samples were invasive. However, only 62.5% (10/16) of isolates originated from poultry have *invA* gene as presented in table 2. No amplified DNA fragments were obtained from *E. coli* K12, the negative control.

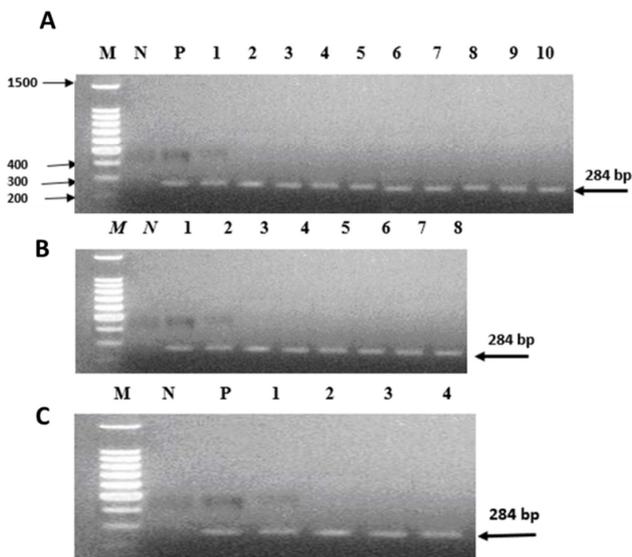


Figure 1. 2% Agarose gel electrophoresis of PCR products of *Salmonella InvA* gene isolated from different different sources of the food. PCR product is 284 bp. The positive samples are from (A) chicken meat, (B) cakes, (C) tarts and cattle meat. Lane M: molecular size marker (1kbp DNA ladder). Lane N: negative control which is genomic DNA of *E. coli* K12. P: positive control (*S. enterica*), numbers of the lanes represent the positive samples.

4. Discussion

The existence of *Salmonella* species in food is harmful to the health regardless the level of contamination, leading to salmonellosis [10]. Invasive non-typhoidal *Salmonella* have been considered as a remarkable agent of bloodstream infection in Africa with mortality rate of 20-25% in both children and adults [15]. Our results showed that the prevalence of *Salmonella* spp among all establishments were (8.11%). High incidence rate of food contamination was reported from poultry butchers (16/55, 29.1%) and the

lowest from the cattle butchers (2/120, 1.7%). The main sources of meat contamination could be the internal organs of animals, fecal materials of workers, and contaminated water. Regarding the cakes and tarts, out of 35 premises investigated, *Salmonella* spp were isolated at overall rate 5.9% and 8.0% respectively as indicated in (table 2). Contamination of these products by *Salmonella* spp threatens the health of consumers as they must not contain any trace of this pathogen [6]. The source of contamination was not recognized in this study; however, it might be originated from the workers, the ingredients such as eggs, raw milk, milk products, and fresh fruits.

Our findings were close to the previous studies in Tripoli where they reported *Salmonella* spp at 7.56% and 8% [1] respectively, and disagreed with the finding obtained by [4] which reported *Salmonella* at 12.9%. Our results were also different than studies conducted in other countries [18, 25, 9, 8, 20]. The inconsistency with other results is probably attributed to the differences in sources of the samples, hygiene practices, and the methods applied in *Salmonella* identification.

The PCR results of *Salmonella* invasive encoding gene; *invA*, verified the results achieved by cultural and biochemical assays in 22 isolates. The target band of *invA* gene was not detected in 6 isolates which means that these strains are not invasive or have other mechanisms for invasion [13]. The primers used in the amplification of *invA* gene contain sequences specific to *Salmonella* genus irrespective of serovar and sample type. Therefore, it has been considered as international marker for identification and characterisation of salmonellosis [13]. Detection of *invA* gene indicate that *Salmonella* isolates are invasive and can cause gastroenteritis illness. This gene is located in *salmonella* pathogenicity island 1 (SPI) and encodes the inner membrane protein, which is necessary for invasion of the epithelial cells [17, 3]. The isolates lack *invA* gene are motile, so harbouring of flagella confers the pathogenicity [22]. Our results of *invA* detection are disagreed with findings of [12] in Egypt (50%), and also with findings of [25] by (12.5%) in Indonesia.

5. Conclusion

According to our knowledge, this study is the first published work that employs *invA* gene investigation in isolated *Salmonella* spp in Libya. As the meat is one of the most consumable foods in Libya, the high incidence rate reported here, particularly from the chicken in addition to detection of virulence specific gene (*invA*) could be have economic and public health implications. Introducing of PCR methods is highly recommended in country to ensure food safety and security, due to their rapidity, accuracy and sensitivity in detection of *Salmonella* spp. Furthermore, performing larger studies on detection of *Salmonella*, identifying the serotypes, examining other virulence genes are important to elucidate the prevalence of this enteropathogenic bacteria in the country, and generalise national database.

Table 1. The primers used in this research.

Name of primer	Sequence (5'-3')	Size of target (bp)	reference
<i>invA</i> -F	GTGAAATTATCGCCACGTTCCGGCAA	284	Baldwin <i>et al</i> (2009)
<i>invA</i> -R	TCATCGCACCGTCAAAG GAACC		

Table 2. Salmonella isolates of various food sources taken from different establishments.

Establishments	Total number of establishments	Food sources	Total number of samples, N=370	Positive samples	Incidence percentages	<i>InvA</i> gene (%)
Confectionery premises	35	Cakes	170	10	5.9%	8 (80)
		Tarts	25	2	8.0%	2 (100)
Poultry butcheries	11	Chicken meat	55	16	29.1%	10 (62.5)
Cattle butcheries	2	Camel meat	120	2	1.7%	2 (100)

Conflict of Interest

All the authors do not have any possible conflicts of interest.

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