Detecation of foodborne microbes in distrbuted eggs within the city of Misurata, Libya

Rasha R. Shaaib[1]; Ibrahim A. Teka[2]; Khdija M. Ahmed[3]

[1] Misurata Academy for Postgraduate Studies

[2] 'Medical laboratory department / Medical Technology College – Misurata

[3] Microbiology Department / Faculty of Medicine – Sebha University

For correspondence:
teka42@hotmail.com

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Abstract

Salmonella and *Shigella* is one of the most important foodborne pathogens worldwide; This study was aimed to investigate the presence of pathogenic *Salmonella* and *Shigella* isolated from eggs; then screening isolates for *stn* gene in *Salmonella* and *invC* gene in *Shigella* using PCR technique. A total of 50 samples of distributed eggs were randomly collected from stores, supermarkets and street vendors around the city. Two *Salmonella* isolates were found in egg samples 4% (2/50) and one *Shigella* was found in egg sample 2% (1/50). All *Salmonella* isolates were positive for the stn gene and *Shigella* was positive for the invC gene. The result confirmed that the transfer of these organisms through the food chain poses imminent danger to the consumer and concluded that there is a need for continuous surveillance of eggs especially the locally produced ones, suggested using PCR to minimize the possibility of human infections with *Salmonella* and *Shigella*.

I. INTRODUCTION

Eggs are nutritious foods and they form an important part of the human diet. However, they are perishable due to improper handling; this food is one the main sources of foodborne pathogens due to high contents of proteins and fat which represent an enriched media for growth and multiplication of pathogens (1).

Several pathogenic bacteria have been isolated from these foods are sometimes found on eggshells and can survive or grow in the edible liquid content (yolk and white) of eggs. Contamination of eggs with these microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce food-borne infection or intoxication to consumers (2; 3).

However, the vast majority of egg-associated human illnesses are attributed to bacteria of the genus *Salmonella* and *Shigella* (4). Globally Salmonella and *Shigella* species remain major contributors to acute enteric infections (5). They are closely related enteric pathogens belonging to the family Enterobacteriaceae and are found naturally in the environment, humans and food (6).

Contamination of foods, especially the eggs, with *Salmonella spp.* and *Shigella* spp. is a major concern for public health.

Therefore the use of a rapid method for *Salmonella* detection in this important food commodities is urgently needed, Established conventional methods to detect and identify *Salmonella* are time consuming and include selective enrichment and plating followed by biochemical tests (7; 8). On the other hand, polymerase chain reaction (PCR) is a rapid and reliable method for detection and identification of foodborne pathogens such as *Salmonella* as a complementary to conventional culture (9; 10).

Several genes have been used to detect *Salmonella* and *Shigella* in natural environmental samples as well as food and faecal samples. *stn* and *invC* gene were a virulence gene. The *stn* gene contains sequences unique to *Salmonella spp*. (11), and *invC* gene contains sequences unique to Shigella spp. (12).

In this study we investigate the presence of pathogenic *Salmonella* and *Shigella* isolated from eggs; then screening isolates for *stn* gene in *Salmonella* and *invC* gene in *Shigella* using PCR technique.

II. MATERIALS AND METHODS

Sample collection

A total of 50 samples of distributed eggs, were collected from randomly selected from stores, supermarkets and street vendors in Misurata, Libya. The egg samples were purchased like consumer and immediately transferred to the laboratory for microbial investigation.

Isolation of bacteria

Salmonella strains were isolated according to the standard ISO-6579 method described by (International Organization for Standardization 1993) (13); with some modification to detection.

Identification of Isolates

Pure colonies of all the isolates were identified using colony morphology, Gram staining, TSI agar test (Biolab, Merck - South Africa), and API-10s test kit (bioMerieux, Inc., France).

Polymerase chain reaction (PCR) for *stn* and *inv*C gene

The positive sample for *Salmonella* and *Shigella* were tested for the presence of virulence associated *stn* gene and *invC* gene.

DNA extraction

Template DNA of samples incorporated in PCR reactions was prepared by boiling and snap chill method (14). Briefly, the samples were rescued from frozen and grown in 10 ml Luria Bertani (LB) broth (Sigma) and incubated at 37°C for 24 hrs. Thereafter, one ml of the test culture was taken in a 1.5 ml tube and centrifuged (Eppendorf Minicentrifuge MiniSpin) at 8000 rpm for 10 min. The pellet was washed twice with sterile saline solution and finally re-suspended in 300 µl sterilized DNAse and RNAse-free milliQ water (Millipore, USA). The suspension was vortexed and heated for 10 min and then were immediately kept on ice. Suspensions were centrifuged at 12000 rpm for 10 min and 3µl of the supernatant was used as a DNA source in PCR mixtures.

Primers set and PCR amplification program

The *Salmonella* specific primers, Stn P1 and Stn M13 (11) have respectively the following nucleotide sequence based on the *stn* gene of *Salmonella*, The *Shigella* specific primers, SgenDF1and SgenDR1 (12) have respectively the following nucleotide sequence based on the *stn* gene of *Salmonella*, as shown in table1, The primers sets of *InvA* genes in this study was synthesized from Invitrogen (USA).

Table 1: Primers used in the study

Primer	Primer Sequence (5'→3')	Target gene
Stn P1	TTG TGT CGC TAT CAC TGG CAA CC	*Salmonella* (*stn*) 617bp
Stn M13	ATT CGT AAC CCG CTC TCG TCC	
SgenDF 1	TGC CCA GTT TCT TCA TAC GC	*Shigella* (*InvC*) 875bp
SgenDR 1	GAA AGT AGC TCC CGA AAT GC	

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ion mixture contained 2.5µl of DNA solution from the procedure above, 5 units of GoTaq reaction buffer (Promega Corp., USA), 1 x PCR reaction buffer, 1.5mM MgCl, 10mM PCR nucleotide mix (Promega Corp.,
USA), and 1.5µM DNA primer in a final volume of 50µl.

The cycle of amplification conditions were as follow: An initial incubation at 95°C for 2min. Followed by 30 cycles for another three steps: denaturing (95°C, 30s), annealing (50°C, 30s), primer extension (72°C, 45s). This was followed by final extension temperature at 72°C for 7min.

Electrophoresis of PCR products

Amplification product sizes were verified by electrophoresis of 10µl samples in a 1.0% agarose (sigma) TBE gel with syber safe (promega).

III. RESULTS AND DISCUSSION

Food is a basic need to support human survival, so food security is an important and increasing focus of attention. Food commodities that are often related to food poisoning cases are eggs, this is because eggs are a good medium for growth of bacteria (15).

In this study a total of 50 eggs were examined for the presence of *Salmonella* and *Shigella* by cultural examination. After incubation, two samples (4%) of eggs were found to be positive for *Salmonella* and One sample (2%) from a total number of examined samples as shown in table2. The level of contamination in this study is lower than a study conducted in Nigeria (16), and higher than a study conducted in Turkey (17).

Table 2: Prevalence of *Salmonella* and *Shigella* in eggs

Sample type	Total	Cultural examination for			
		Salmonella		*Shigella*	
		+ve	-ve	+ve	-ve
Eggs	50	2 (4%)	48	1 (2%)	49

The higher prevalence of *Salmonella* and *Shigella* spp. possibly due to lack of vaccine and differences in prevention and control strategies, and possibly due to environmental contamination Thus, this study reports the contamination of contents of eggs by *Salmonella* and *Shigella* species but quantification of

such contamination should be further investigated.

In order to confirm the former results, PCR assay was carried out for the detection of the *stn* gene and *invC* gene using a specific primer to confirm the presence of *Salmonella* and *Shigella* isolated from eggs samples.

The PCR which performed for *stn* (617bp fragment) gene resulted positive amplifications in all Salmonella isolates (100%) as shown in (Figure 1)

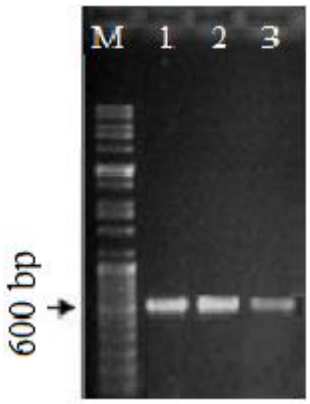

Figure 1: PCR amplification of *stn* gene for *Salmonella* samples at correct size of 617bp

Lane M: Molecular ladder, Lane 1 & 2: represents *salmonella* sample, lane 3: *stn* gene positive control

The PCR which performed for *invC* (617bp fragment) gene resulted positive amplifications in *Shigella* isolates (100%) as shown in (Figure 2)

Thus, this study support other research that recommendation to use *stn* gene as target to dedicated *salmonella* spp , and use *inv*C gene as a target to detection *Shigella* spp. (12; 18); meanwhile confirmed that the transfer of these organisms through the food chain poses imminent danger to the consumer.

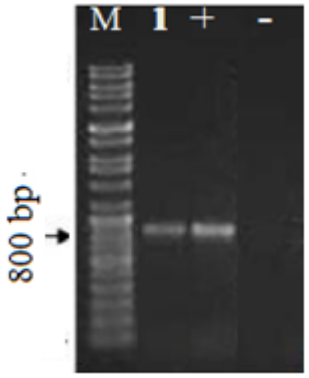

Figure 2: PCR amplification *of inv*C gene for *Shigella* samples at correct size of 875bp

Lane M: Molecular ladder, Lane 1: represents *Shigella* sample, Lane +: *inv*C gene positive control, Lane -: *inv*C negative control

IV. CONCLUSION

The detection of *Salmonella* and *Shigella* in eggs demonstrates that there is source of contamination in farms this may be referred to hen's droppings and contaminated litter. Therefore, it is important to apply new techniques and educate those in direct contact with the hens in order to control *Salmonella* and *Shigella* transmission in farms; as well as, using an efficient and rapid detection method to monitor and control Salmonella and *Shigella* is of great significance for ensuring food safety.

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