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Detection of Salmonella in Meat Products by Polymerase Chain Reaction

George, Armany[1], Hemmat, Ibrahim[2], Reham, Amin[2], Naglaa, Hagag[1]

[1]*Genome Research Unit., Animal Health Research Institute, Dokki, Egypt*

[2]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*

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ABSTRACT

A total of 100 different meat samples 50 grams from each sample were chosen randomly including raw meat products as minced meat and sausage and treated meat products as luncheon and basterma (25 samples from each) collected from several markets in two regional Governorates (Cairo and Giza). Traditional bacteriological method and polymerase chain reaction (PCR) tool were used to look for salmonella in these samples. Bacteriological data demonstrated the occurrence of 16%, 8%, 0% and 0% in minced meat, sausage, luncheon and basterma, respectively. PCR validation for identifying of salmonella by *(invA)* gene showed the same conformance of results in prevalence of that of the bacteriological isolation where it was 16%, 8%, 0% and 0% in minced meat, sausage, luncheon and basterma, respectively. Traditional bacteriological method can take two to three days to show such results and up to seven to ten days to validate them, while polymerase chain reaction proved and it is reliable, less labor, accurate and requires less effort and time comparing to traditional method.

1. INTRODUCTION

Salmonella is one of the most popular bacterial pathogens that cause gastroenteritis and has been linked to many outbreaks worldwide. Infection by salmonella is found all over the world, and outbreaks have been connected with a wide range of foods, making them a major public health concern (CDC, 2010).

Meat and its products are highly perishable and can spoil easily and soon become inappropriate to eat and perhaps harmful to one's health due to microbial growth of endogenous enzymes change the chemical composition and break it down (Judge, 1990).

Microbiological testing is critical for determining food safety and quality. In the past, culture methods were primarily used to detect and identify microorganisms. These procedures are the most accurate and trustworthy for detecting foodborne pathogens, but they are time-consuming, take a long time to process, and are expensive. The main downside of this procedure is that it might take up to two days for any results to materialize (Jasson et al., 2010).

Polymerase chain reaction (PCR) is a potent technology that has transformed molecular biology research. It may be used to diagnose microbial infections, genetic illnesses, and pathogens in food samples (Huang et al., 2009).

2. MATERIAL AND METHODS

2.1. Collection of samples

Actually, 100 samples of raw meat and cooked meat (minced meat, sausage, luncheon and basterma) were gathered from several sources in Cairo and Giza Governorate markets. Fifty grams from each sample were submitted to the lab in Animal Health Research Institute for bacteriological examination in an icebox under complete aseptic conditions.

2.2. Sample preparation

Accurately, 25 grams of each sample were taken under aseptic conditions into blender to which (225ml) peptone water (0.1%) was included. After that, the blender was set at 3000 rpm for no more than 2.5 minutes to get 10^{-1} dilution, after that (1ml) from the original solution was transferred into separate tube containing (9 ml) peptone water from which tenfold serial dilutions were prepared (ICMSF, 1978).

2.3. Bacteriological isolation

According to ISO. 6579 (2002), One ml of the pre-enrichment culture was infused into ten ml Rappaport Vassilidis broth as a selective enriched broth and incubated for 24± 2 hrs at 41.5 ºC ± 1ºC. Then the surface of the previously prepared Xylose Lysine Desoxycholate (XLD) agar was streaked with a loop of the selective enriched broth. The inoculation plates were kept at 37°C ± 1°C for 24 hours.

2.4. Optimization of the PCR

DNA extraction and purification direct from the meat products where purified DNA was obtained from samples using a Thermo Scientific Genomic DNA extraction kit. Table (1) illustrated the Oligoneucleotide primers utilized for amplification of the salmonella invasion *(invA)* gene, which were defined using integrated DNA technology. The primers were lyophilized and re-suspended in sterile water to a concentration of 100 pmol/l. These primers are thought to amplify a 284-bp region. The reaction is 6.25 µl Verso mastermix (2x), 0.75 µl PCR grade water, 1.5 µl forward

* Corresponding author: georgearmany006@gmail.com

primer, 1.5 µl reverse primer, 2.5 µl DNA to obtain a final volume of 12.5 µl in the reaction, according to Verso mastermix (Thermo Scientific).
Amplifications conditions for thermocycling were 95˚C for 5 min for primary denaturation and followed by 40 cycles of heat denaturation at 95˚C for 15 sec, primer annealing at 59 ˚C for 45 seconds and DNA extension at 72˚C for 1 min, this was followed by final extension at 72˚C for 10 min.
After PCR reaction, 5µl of the PCR product was resolved on 1.5% agarose gel. In a gel documentation system, the gels were stained with Ethidium bromide (0.2g/ml) and photographed with UV transillumination (BioRad) (Sambrook et al., 1989).

Table 1 Oligonucleotide primers of salmonella (invA) primers used

Target pathogen	Target gene	Primer	Sequence (5` - 3`)	Amplicon size bp	Reference
salmonella	*invA*	Forward Reverse	GTGAAATTATCGCCACGTTCGGGCAATCATC GCACCGTCAAAGGAACC	284	*Rahn et al., (1992)*

3. RESULTS

3.1. Bacteriological Finding

Plates prepared by Xylose Lysine Desoxycholate (XLD) agar have been tested for suspected colonies of salmonella that appear red with black centers or without. Table (2) showed the finding of salmonella isolation, which revealed that the frequency of salmonella was detected from 25 samples from each meat product. The results in raw products were detected in 4 samples for minced meat and in 2 samples for sausage. While salmonella failed to be detected in cooked products as luncheon, and basterma. Prevalence was 16 %, 8 %, 0 %, 0 %, respectively from each product. Based on The Egyptian Organization for Standardization & Quality (EOSQ). 1694 (2005), meat products must be salmonella-free.

Table 2 Prevalence of salmoenlla in examined meat product samples

Types of Samples	Number of examined samples	Bacteriological finding	
		No. of positive samples	%
Minced meat	25	4	16%
Sausage	25	2	8%
Luncheon	25	0	0%
Basterma	25	0	0%
Total	100	6	6%

3.2. PCR Result

The positive random bacteriological samples were re-examined for confirmation by PCR, as shown in Figure (1) showed the marker (100bp), lane 1-4 showed 4 positive minced meat samples for salmonella (*invA* gene 284bp), lane 5-6 showed 2 positive sausage samples for salmonella (*invA* gene 284bp), negative Control and the positive control for salmonella (*invA* gene 284bp). The PCR result of salmonella for detection of meat products by using *(invA)* gene with 40 cycles of heat denaturation at 95˚C for 15 sec, primer annealing at 59 ˚C for 45 sec and DNA extension at 72˚C for 1 min, this was followed by final extension at 72˚C for 10 min. The results of PCR showed the same finding of bacteriological isolation where positive results were detected in 4 samples of minced meat and 2 samples of sausage, while cooked meat samples showed negative results.

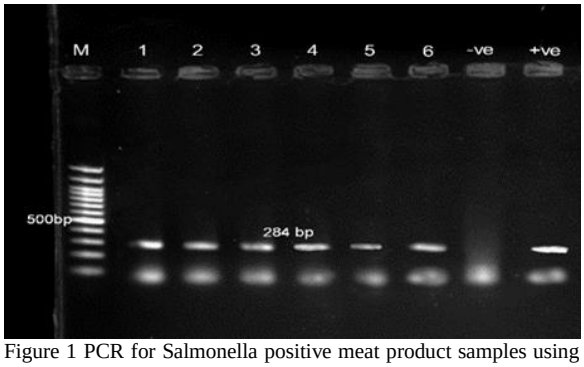

Figure 1 PCR for Salmonella positive meat product samples using (invA) primers. M: marker (100bp), Lane 1-4: 4 positive minced meat samples for Salmonella (*invA* gene 284bp), Lane 5-6: 2 positive sausage samples for *Salmonella* (*invA* gene 284bp), –ve: Negative Control, +ve: Positive Control for *Salmonella* (*invA* gene 284bp).

4. DISCUSSION

Salmonella is still a serious public health concern pathogen. Meat products are important vehicles for transmission of salmonella in food (Gashe et al., 2006). In this study a total of 100 random samples of meat products; minced meat and sausage (which represent raw meats), luncheon and basterma (which represent cooked meats) were examined bacteriologically to reveal the prevalence of salmonella and the prevalence was 16%, 8%, 0% and 0%, respectively.The obtained results of the examined minced meat samples were nearly similar to Raseta et al. (2017), while higher than those obtained by Terentjeva et al. (2017) (1.5%), and lower than those of Hassanein et al. (2011) (20%).

In the examined sausage samples, the results were nearly similar to those of Raseta et al. (2017) (7%), while higher than those obtained by Abd El-Atty and Meshref (2008) (4%), and lower than those of Samaxa et al. (2012) (25.3%).

On the other hand, the obtained results of the examined luncheon samples were nearly similar to those of Darweesh (2008) and Saad et al. (2018) (0%) who failed to detect salmonella in luncheon meat products.
Furthermore, the obtained results of the examined basterma samples were nearly similar to those of Darweesh (2008) (0%) who failed to detect salmonella in basterma products.
Foodborne salmonellosis is often associated with the ingestion of raw meat products, typically caused by tainted animals used in the processing of foodstuffs, or by infection of carcasses or edible organs. The illness began with the consumption of meat infected with sufficiently large amounts of bacteria, resulting in the invasion of the small intestine (Laufer et al., 2015).
The significance of not detecting salmonella in cooked products may be due to the high cooking temperature which plays a big role in killing most of food pathogens. Also, the usage of pickling and spices agents in these treated products can affect the viability of these pathogens (Zin et al., 2017).
The result of optimization for validation of PCR on salmonella was compatible with the bacteriological finding concerned in this study. This result was similar to the results of Can et al. (2014); Sunar et al. (2014); Nguyen et al. (2016); Sharma (2016) and El-Sayed et al. (2019) who used (*invA)* gene in the detection of salmonella in food products.

PCR result proves the accuracy of this technique to be used side by side with the bacteriological traditional method.

5. CONCLUSION

PCR methods offer a sensitive and specific detection of salmonella. It is rapid, easy to handle and constitute very valuable tool for routine applications comparing to the bacteriological traditional method.

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