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Isolation and molecular identification of *Salmonella serovar from fermented foods*

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ABSTRACT

The present investigation was planned to detect the incidence of *Salmonella* serovar in some fermented foods. The examined fermented foods were represented in 100 samples as 20 yoghurt (10 Baladi & 10 commercial), 20 Rayeb (10 Baladi & 10 commercial), 20 Bramily cheese, 20 pickled cucumber and 20 salted fermented fishes (10 Molouha & 10 Fesikh). The samples were collected randomly from different localities in Assiut city, Egypt. All the samples were subjected to the protocol of *Salmonella* isolation, in which, a weight of 25 g of the sample was taken into 225 ml of a preenriched broth of buffered peptone water (BPW) 1% and incubated at 37° C for 24 h, then 1 ml of the pre-enriched broth was transferred into an enriched broth of Rappaport-Vassiliadis (RV) broth and incubated at 40° C for 48 h, after that a loopful was inoculated onto Salmonella Shigella (S.S.) agar media for colorless colonies with a black center, which suspected to be *Salmonella*. The suspected *Salmonella* isolates were biochemical identified using triple sugar iron agar test, lysin iron agar test and urease test, then molecular identified using PCR. The obtained results showed that 9% of the examined samples were contaminated with *Salmonella* and were represented only in the examined fermented fish samples as 4 Molouha samples and 5 Fesikh samples, while all the rest samples were *Salmonella* free. *Salmonella Enteritidis* was isolated from the 4 Molouha samples, while, *Salmonella* serovar *Typhimurium* could not isolated from the all samples. The present study recommended the application of strict hygienic measures during the food production and especially for fermented fishes and according to the food safety management system (FSMS).

INTRODUCTION

One of the first biotechnologies for producing food products with desirable qualities, such as a long shelf life and good organoleptic features, is fermentation [1].

Fermented foods are foods or beverages made through regulated microbial growth and enzymatic production of major and minor nutritional components.
Food preservation has been done for millenia using fermented dairy products, which were among the first "processed" foods consumed by humans [2].
Although being rich in nutrients, fresh milk and its fermented products provide an ideal environment for the growth of a wide range of bacteria, aiding in the spread of milk-borne diseases such as salmonellosis, brucellosis, TB, shigellosis, and staphylococcosis [3].

In Egypt, pickles are a popular ready-to-eat food. However, due to the fact that the items are not thermally sterilised before consumption, there is considerable concern regarding their safety. Food handlers' lack of food hygiene education and comprehension contributed to contaminated conditions in the food supply, food processing facilities, and the selling environment [4].

One of the most ancient methods of preserving fish is the salting process, which is still used in a number of places all over the world. Products manufactured from salted fish are widely used all throughout the world, and even modern countries have discovered them to be safe for centuries. [5, 6].
Traditional Egyptian salted fish known as fesikh has long been a staple of the Egyptian cuisine, particularly during important occasions like spring day. Additionally, sodium chloride is a flavour enhancer due to its impact on several biochemical pathways, which includes its ability to decrease or increase the enzymatic activity of particular enzymes involved in the creation of various organoleptic characteristics. [7].
Salmonella can be spread during storage and processing of fish and shellfish as well as via contaminated waters [8].

One of the most common foodborne pathogens that affects people and causes foodborne illnesses in all developed and developing countries is salmonellosis [9].
Even at low contamination levels, milk fermentation can allow Salmonella to persist.by Savran et al. (2017) [10].

To avoid risks to human health, it's critical to identify Salmonella germs on food products as soon as possible. Till now, about 2500 distinct species of Salmonella have been discovered. Salmonella enterica serovar Typhimurium (Sal. Typhimurium) and Salmonella enterica serovar Enteritidis (Sal. Enteritidis) are the most common causes of foodborne illness. Because of the substantial number of Salmonella serovars, the best early detection look at is nucleic acid based. These approaches employ a specific Salmonella DNA sequence, resulting in an accurate identification. Furthermore, adopting highly advanced real-time PCR technology to automate DNA extraction, amplification, and detection might improve the detection method. [11].
Therefore, the current work was carried out to study the incidence of *Salmonella* spp. in some fermented foods for raising food safety concern; Moreover, for molecular characterization of the isolated *Salmonella* spp.

MATERIALS AND METHODS

Collection of samples:

100 fermented food samples were represented as 20 yoghurt (10 Baladi & 10 commercial), 20 Rayeb (10 Baladi & 10 commercial), 20 Bramily cheese, 20 pickled cucumber and 20 salted fermented fishes (10 Molouha & 10 Fesikh) were collected randomly from Assiut city, Egypt. The samples were transferred directly to the lab for investigation under aseptic precautions.

Isolation of *Salmonella* [12, 13]**:**

225 ml of buffered peptone water (BPW) 1% were used to pre-enrich 25 g of the sample before it was incubated at 37 °C for 24 hours. After that, 10 ml of Rappaports-Vassiliadis (RV) enriched broth were added to a tube with 1 ml of the pre-enriched broth, and the tube was incubated at 40° C for 48 hours.

A loopful from the incubated enriched broth was streaked on Salmonella-Shigella (S.S.) agar selective plates. The plates were incubated at 37° C for 24 h. for colorless colonies with black centres that were suspected to be Salmonella. The suspected Salmonella colonies were cultured on nutrient agar slope for further identification using biochemical screening tests as sugar fermentation, H2S production on triple sugar iron agar, lysine decarboxylation, urea splitting ability in Christensen's urea agar [14].

Molecular detection of *Salmonella*:

The multiplex PCR- based assay (m-PCR) using 3 sets of primers was developed for *Salmonella* spp. detection and the most common serotypes of *Salmonella* enterica as *Sal. Enteritidis* and *Sal. Typhimurium*. This is a very rapid and simple molecular method for serotyping common *Salmonella*, the specific sequence could be detected in all *Salmonella* enterica serotypes.

Pure colonies were subjected to identification using m-PCR for detection of *Sal. Enteritidis* and *Sal. Typhimurium* that was done in the Molecular Biology Unit of the Molecular Biology Researches and Studies Institute (MBRSI), Assiut University, Egypt.

Table 1. Primers for multiplex-PCR identification of the most frequent *Salmonella*

Primer	Length (nucleotides)	Primer sequence (from 5 to 3)	Size	Reference
ST 11-F (1)	24	F: GCC AAC CAT TGC TAA ATT GGC GCA	429	[15]
ST 15-R (1)	25	R: GGT AGA AAT TCC CAG CGG GTA CTG G		
S1-F (2)	20	F: GCC GTA CAC GAG CTT ATA GA	250	[15]
S4-R (2)	20	R: ACC TAC AGG GGC ACA ATA AC		
Fli15-F (3)	22	F: CGG TGT TGC CCA GGT TGG TAA T	559	[16]

Tym	22	R: ACT CTT GCT GGC GGT GCG ACT T

As shown in Table 1, the primers' sets used were ST11 and ST15 as a universal gene for Salmonella spp., SI and S4 for Sal. Enteritidis, and Fli15 and Typ04 for Sal. Typhimurium according to Soumet et al. (1999) [15, 16].

The protocol used for boiling extraction of genomic DNA from the cells was performed according to Schmitt and Pawlita (2009) [17]**.**

The 22µ l multiplex PCR reaction contains 11µ l of the PCR master mix, 1µ l of each primer, and 5µ l of the extracted DNA. The first stage of denaturation was performed at 95° C for 5 min, then there were 35 cycles of 95° C for 1 min, 48° C for 1 min, and 72° C, with the final extension was performed at 72° C for 10 min. after amplification, 1% agarose was utilised for electrophoresis.

Agarose-gel electrophoresis:
Using the appropriate molecular weight markers, agarose gel electrophoresis was used to check the PCR results for evidence of positive amplification. [18].

RESULTS

Table 2. *Salmonella* incidence in the examined fermented food samples

The examined samples	**The samples No.**	**Presumptive isolated *Salmonella***		**PCR confirmed *Salmonella***		***Sal. Enteritidis***		***Sal. Typhimurium***		**Other *Salmonella* spp.**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Baladi yoghurt	10	-	0	-	0	-	0	-	0	-	0
Commercial yoghurt	10	-	0	-	0	-	0	-	0	-	0
Baladi Rayeb	10	-	0	-	0	-	0	-	0	-	0
Commercial Rayeb	10	-	0	-	0	-	0	-	0	-	0
Bramily cheese	20	-	0	-	0	-	0	-	0	-	0
Pickled	20	-	0	-	0	-	0	-	0	-	0

cucumber											
Molouha fish	10	5	50	4	40	4	40	-	0	-	0
Fesikh fish	10	5	50	5	50	-	0	-	0	5	50
Total	100	10	10	9	9	4	4	-	0	5	5

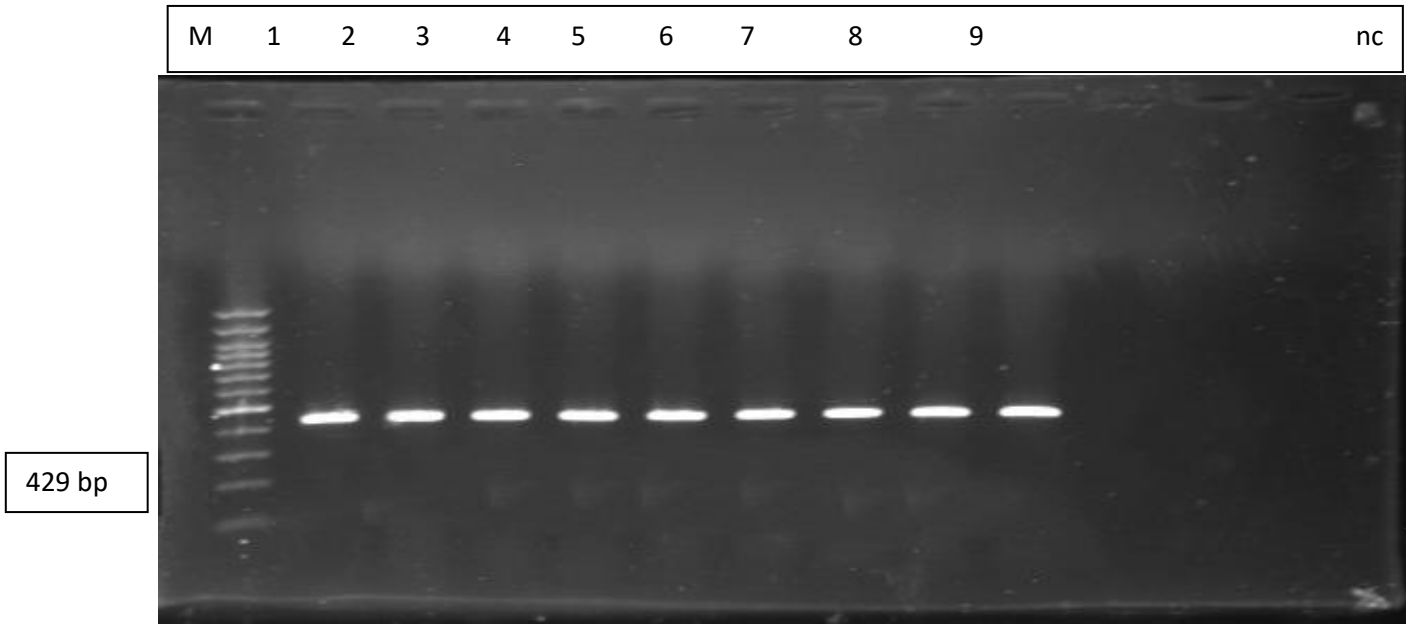

Figure 1. Agarose-gel electrophoresis of the obtained PCR product for the isolated *Salmonella* spp.

M: 100 bp for DNA marker

Lanes 1 to 7 showed positive amplification of 429 bp fragments of *Salmonella* spp.

nc: negative control

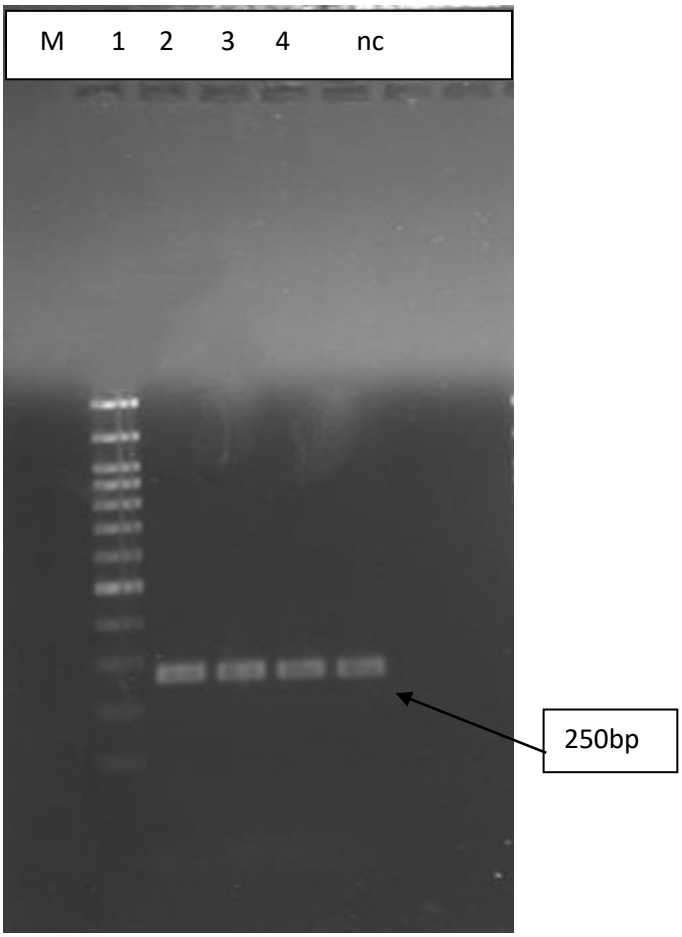

Figure 2. Agarose-gel electrophoresis of the obtained PCR product for the identified *Sal. Enteritidis*

M: 100 bp for DNA marker

Lanes 1 to 4 showed positive amplification of 250 bp fragments of *Sal. Enteritidis*

nc: negative control

DISCUSSION

For those in developing countries without access to probiotics, the nutritional advantages of eating fermented foods may be particularly significant. In addition to providing probiotics and reducing diarrhea, these fermented foods can strengthen the immune system's capacity to combat other germs. Ineffective manufacturing practises increase the danger of microbial contamination of food items, despite the many benefits

of fermentation processes involving a variety of bacteria, including beneficial ones. This phenomenon is particularly evident in underdeveloped countries when fermented food production process are either too geographically scattered and individualized or lack standards and supervision groups. In this circumstance, the risk of consumption of fermented foods should be considered, particularly if they have been contaminated with pathogenic microbes such as viruses(19) . therefore, routine Salmonella detection in fermented foods is an important aspect of public health programs.

However, the prevalence of Salmonella in fermented milk products in the current study (Table 2) was similar to that report by Mohammed (2019) (20)

that revealed that no *Salmonella* was detected in yoghurt obtained from local markets and super markets.

Samet-Bali *et al*. (2016), Beukes *et al*. (2001), Abdel-Rahman *et al*. (2009) [21, 22, 23] showed that no *Salmonella* was detected in Rayeb.

Salmonella could not be detected in Domietti cheese by Hassan & Gomaa (2016) and Mehmood *et al*. (2020) [24, 25]. El Bagoury *et al*. (2019) [26] revealed that *Salmonella* species could not be detected in both Kariesh or Domiati cheeses.

Higher result was obtained by Amin and El-Sherif (2018) [27] as *Salmonella Typhimurium* was isolated from 10% of yoghurt. Namaei *et al*. (2015) [28] studied the antibacterial activity of 30 various non-industrial yoghurt on *Shigella* and *Salmonella Enteritidis* isolates and the results showed that neither of non-industrial yogurts had an effect on higher than 51.5% of isolates.

Ibrahim *et al*. (2015) [29] revealed that *Salmonella* spp. has been isolated from 4% of Domiati cheese.

Vegetables which often constitute the raw material for pickled foods can carry pathogenic microorganisms like *E. coli* and *Salmonella* (Matthews, 2017) [30]. The presence of *Salmonella* and thermotolerant coliforms in ready-to-eat vegetables were reported by Oliveira *et al*. (2011) [31].

In the present study, there was no *Salmonella* detected in pickled cucumber (Table 2). Similar result was showen by Chien *et al*. (2023) [32]

that revealed that no *Salmonella* spp. was detected in Pickles. Higher result was revealed by Muramatsu *et al*. (2020) [4], in which, 41.7% of pickles was contained *Salmonella*.

Salmonella has been found in fish and fisheries products despite the fact that it is not psychotropic and is not endemic to the watery environment. [33].

The FDA documented that *Salmonella* was the most common contaminant of fish & fishery products [34].

Edris *et al*. (2017) showed that no *Salmonella* was detected in salted fishes Mugil cephalus & sardine [35]. Moreover, Basti *et al*. (2004) showed that heavy-salted fish & heavy-cold smoked fish were negative for *Salmonella* [36].

CONCLUSION

Salmonella was found in feremented salted fishes, Molouha and Fesikh, but could not isolated from fermented milks and pickled cucumber. *Salmonella Enteritidis* was detected only in Molouha, while *Salmonella Typhimurium* could not isolated from all fermented products.

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