

## Original Article

# Prevalence and molecular characterization of *Salmonella* serovars in milk and cheese in Mansoura city, Egypt

Amira Hussein El-Baz, Mohammed El-Sherbini, Adel Abdelkhalek and Maha Abdou Al-Ashmawy

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## AFFILIATIONS

- Amira Hussein El-Baz
  - Mohammed El-Sherbini El-Sayed
  - Adel Abdelkhalek
  - Maha Abdou Al-Ashmawy
- Food safety Department of Food Hygiene & Control Faculty of Veterinary Medicine, Mansoura University, Egypt.

## ABSTRACT

**Objective:** This study was conducted to determine the prevalence of *Salmonella* in milk (farm bulk milk, raw market milk) and cheese (kareish, white soft cheese) samples that were collected randomly from farms, supermarkets, small vendors and shops in different districts of Mansoura city, Egypt.

**Materials and methods:** A total of 100 farm bulk milk, raw market milk, kareish cheese and white soft cheese samples (25 of each) were screened for the prevalence of *Salmonella* spp. The *Salmonella* isolates were isolated and identified by conventional bacteriological techniques, which were further confirmed genetically by polymerase chain reaction (PCR) based on the presence of *invA* gene. Finally, the isolates were serotyped.

**Results:** *Salmonella* could be detected in 15% (n=15/100) samples with a prevalence of 12% (n=3/25), 24% (n=6/25), 20% (n=5/25) and 4% (n=1/25) in raw market milk, raw farm bulk milk, kareish cheese and white soft cheese, respectively. The *Salmonella* isolates were serotyped into *S. enteritidis* 33.3% (n=9/27) which was the most frequent, followed by *S. typhimurium* 25.9% (n=7/27), *S. heidelberg* 14.8% (n=4/27), *S. infantis* 11.11% (n=3/27), *S. tsevia* 11.11% (n=3/27) and *S. haifa* 3.7% (n=1/27).

**Conclusion:** The present study confirms the presence of *Salmonella* in milk and cheese samples in Mansoura, Egypt, indicating that the dairy products can act as potential sources of *Salmonella* infection. Thus, appropriate hygienic measures are suggestive for combating Salmonellosis in Egypt.

## CORRESPONDENCE

**Adel Abdelkhalek**  
Professor of Food safety, Head  
of Department of Food Hygiene & Control  
Faculty of Veterinary Medicine, Mansoura  
University, Egypt.  
E-mail: [abdelkhaleka@hotmail.com](mailto:abdelkhaleka@hotmail.com)

## KEYWORDS

Salmonella; PCR; *invA*; Serotypes

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## INTRODUCTION

Milk is considered as a high quality and nutrition-rich food for human beings ([Theresa and Nicklas, 2003](#)); this high quality is mainly referred to its composition and hygiene that are provided during the production and storage of milk. However, if the hygienic conditions are not applied properly, the milk can be contaminated by microorganisms leading to its early spoilage ([Oliver et al., 2005](#); [Nanu et al., 2007](#)).

*Salmonella* are the most common pathogenic bacteria in humans and animals causing Salmonellosis. The disease is caused by numerous serovars of *Salmonella*, and this disease can be manifested as mild food poisoning to severe enteric fever ([Jones et al., 2004](#)). When animals are infected with Salmonellosis, high economic loss occurs due to increased mortality and morbidity, and this diseased animals may act as the source of infections for humans through direct or indirect contacts with animals ([Libby et al., 2004](#)). Contamination of the environment and food with *Salmonella* mainly happens by the fecal wastes of the infected animals and humans ([Ponce et al., 2008](#)) as *Salmonella* colonizes mainly in the gastrointestinal tract ([Abulreesh 2012](#)).

The present study was carried out to investigate the prevalence of *Salmonella* in milk and cheese, and reveal out the serotypes of the *Salmonella* isolates.

## MATERIALS AND METHODS

The technique that was used for samples collection was recommended by American Public Health Association ([APHA 1992](#)). One hundred samples (25 each of market raw milk, farm bulk tank milk, kareish cheese and white soft cheese) were obtained from different localities of Mansoura from the period between May 2014 to May 2015. The collected samples kept in an insulated ice box ( $4\pm 1^{\circ}\text{C}$ ) to be transferred to laboratory for bacteriological examination.

**Ethical statement:** The investigation was carried out according to the animal welfare code in Egypt.

**Bacteriological examination:** The isolation and identification of *Salmonella* spp. were done according to ISO 6579 ([ISO, 2002](#)). 25 mL/gm of the samples were transferred to 225 mL sterile buffered peptone water (Oxoid, CM 0509), and mixed well to obtain 10 fold dilution, then 0.1 mL of buffered peptone water cultured broth was aseptically inoculated into 10 mL of Rappaport Vassiliadis (RV) broth (Oxoid, CM 669). The culture was incubated at  $41^{\circ}\text{C}$  for 24 h. A loopful cultured RV broth was streaked onto Xylose-Lysine-Desoxycolate (XLD)

agar (Oxoid, CM 0469) and incubated at  $37^{\circ}\text{C}$  for 18-24 h. The suspected colonies (pink colonies or red colonies with or without black centers) were picked up and subcultured again onto XLD agar plates (for purification) and then incubated at  $37^{\circ}\text{C}$  for 18-24 h. The purified colonies were subcultured onto nutrient agar slants (Oxoid, CM 0003) and incubated at  $37^{\circ}\text{C}$  for 24 h for further identification (biochemical, serological and molecular). All the suspected colonies were biochemically identified on the basis of Indole test, Methyl Red test, Voges proskauer test, Citrate utilization test, Triple Sugar Iron agar test and Urea Hydrolysis test.

**Molecular identification:** The primarily identified *Salmonella* were confirmed by PCR based on the presence of *invA* gene. Extraction of DNA was performed by thermal cell lysis of suspected strains ([Reischl et al., 2002](#)). The DNA was used as template for the amplification of the highly conserved region of *invA* gene using the primers (forward: 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3'; reverse: 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3') specific for all *Salmonella* serovars ([Chiu and Ou, 1996](#)). PCR was performed in a 25  $\mu\text{L}$  reaction mixture with 2x PCR master mix solution (i-Taq™). Cycling conditions were optimized at initial denaturation at  $95^{\circ}\text{C}$  for 4 min, 35 cycles of amplification using thermocycler (Gene Amp PCR system 2700, Applied Biosystems, Foster City, CA, USA) was done with the conditions as denaturation at  $95^{\circ}\text{C}$  for 30 Sec, annealing at  $57^{\circ}\text{C}$  for 45 Sec and extension at  $72^{\circ}\text{C}$  for 1.5 min, followed by final extension at  $72^{\circ}\text{C}$  for 5 min. Vivantis Company 100-bp Plus DNA Ladder (product no. NL1407) was used. PCR products were electrophoresed in 1.5% agarose gel stained with ethidium bromide 0.5 mg/mL ([Sambrook et al., 1989](#)). The DNA band was visualized by gel documentation system.

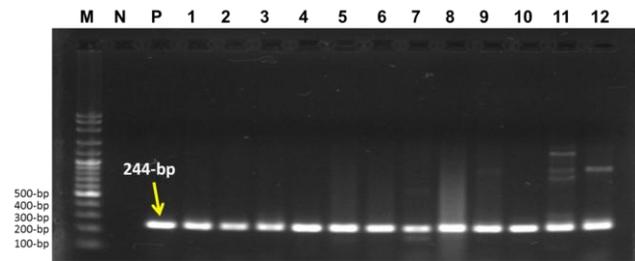
**Serological identification:** The confirmed *Salmonella* isolates were serotyped by slide agglutination with O polyvalent, monovalent antisera (DENKA SEIKEN Co., Japan) and tube agglutination method with H polyvalent antisera of both phase 1 and 2 for the determination of the complete antigenic formula of the isolates, as indicated by [Sambrook et al. \(1989\)](#).

## RESULTS AND DISCUSSION

Milk and milk products are important sources of *Salmonella* especially for the consumers who preferred to consume raw milk. Many factors such as improper hygienic conditions in the farm, food handlers, and consumption of raw milk and milk products are the sources of *Salmonella* infections ([Karshima et al., 2013](#)).

**Table 1:** Prevalence of *Salmonella* in milk and cheese samples

Type of samples	Examined samples	Positive samples (%)
Raw market milk	25	3 (12)
Raw farm bulk milk	25	6 (24)
Kareish cheese	25	5 (20)
White soft cheese	25	1 (4)
<b>Total</b>	<b>100</b>	<b>15 (15)</b>



**Figure 1:** Agarose gel electrophoresis for PCR amplification of *Salmonella invA* gene product (244-bp). M: DNA marker (gene ladder 100-bp), P: positive control, N: negative control, Lane 1 to 12: positive samples.

**Table 2.** Serological typing of *Salmonella* isolates

SL	Infected product	No. of isolates	Identified strains	Group	Antigenic structure	
					O	H
1	Market raw milk	1	<i>Salmonella enteritidis</i>	D1	1,9,12	g,m : -
2		1	<i>Salmonella typhimurium</i>	B	1,4,5,12	i : 1,2
3		1	<i>Salmonella tsevie</i>	B	4,5	i : e,n,z15
4	Farm bulk tank milk	2	<i>Salmonella heidelberg</i>	B	4,5,12	r : 1,2
5		3	<i>Salmonella enteritidis</i>	D1	1,9,12	g,m : -
6		2	<i>Salmonella typhimurium</i>	B	1,4,5,12	i : 1,2
7		1	<i>Salmonella infantis</i>	C1	6,7	r : 1,5
8	Kareish cheese	1	<i>Salmonella infantis</i>	C1	6,7	r : 1,5
9		2	<i>Salmonella typhimurium</i>	B	1,4,5,12	i : 1,2
10		1	<i>Salmonella haija</i>	B	1,4,5,12	Z10: 1,2
11		1	<i>Salmonella enteritidis</i>	D1	1,9,12	g,m : -
12	White soft cheese	2	<i>Salmonella heidelberg</i>	B	4,5,12	r : 1,2
13		2	<i>Salmonella typhimurium</i>	B	1,4,5,12	i : 1,2
14		4	<i>Salmonella enteritidis</i>	D1	1,9,12	g,m : -
15		2	<i>Salmonella tsevie</i>	B	4,5	i : e,n,z15
16		1	<i>Salmonella infantis</i>	C1	6,7	r : 1,5

In the present study as shown in **Table 1**, the prevalence of *Salmonella* was 15%(n=15/100). This finding was similar to the report of [Gwida and Al-Ashmawy \(2014\)](#). *Salmonella* spp. was isolated from 3(12%) raw market milk samples, which is supported by the report of [Karshima et al. \(2013\)](#). Higher prevalence rates of *Salmonella* was recorded by [Ibrahim et al. \(2015\)](#), in contrast, low rate was reported by [Soyutemiz et al. \(2000\)](#), [Chye et al. \(2004\)](#) and [O'Donnell \(\(2007\)](#). Moreover, several studies recorded that *Salmonella* was not detected in milk samples ([Ekici et al., 2004](#); [D'Amico et al., 2008](#); [Zeinhom and Abdel-Latef 2014](#); [Elbagory et al., 2015](#)). This variation might be due to the difference in hygienic practices during milking.

The prevalence of *Salmonella* was 24%(n=6/25) in the farm bulk milk samples as shown in **Table 1**. This result was closely related with the result reported by [Van Kessel et al. \(2011\)](#); however, lower results were recorded in many researches ([Jayarao and Henning, 2001](#); [Warnick et al., 2003](#); [Van Kessel et al., 2004](#); [Karns et al., 2005](#);

[Jayarao et al., 2006](#); [Van Kessel et al., 2008](#)). Higher prevalence rate of *Salmonella* was reported by [Addis et al. \(2011\)](#). However, [Zeinhom and Abdel-Latef \(2014\)](#) failed to detect *Salmonella* in farm bulk milk. This variation might be due to the difference in hygienic and sanitation practices during milking.

**Table 1** showed that *Salmonella* spp. were detected in 20%(n=5/25) of the examined kareish cheese samples. This result was nearly in agreement with [Mohammed et al., \(2013\)](#). However, very low prevalence of *Salmonella* in kareish cheese was reported by [Elbagory et al. \(2015\)](#) and [Ibrahim et al. \(2015\)](#), while [El Sayed et al. \(2011\)](#) and [Nosir et al. \(2014\)](#) failed to detect *Salmonella* in the kareish cheese samples. The results of our study on kareish cheese samples indicated that sanitation practices during manufacture and handling of cheese were insufficient. And, most of these cheese were sold as uncovered condition and without any container. Thus, the risk of contamination with *Salmonella* spp. was high.

In case of white soft cheese, the prevalence of *Salmonella* was 4%(n=1/25) (**Table 1**). Our results were similar with the report of [Ibrahim et al. \(2015\)](#), while [El Sayed et al. \(2011\)](#) reported lower prevalence of *Salmonella* in white soft cheese. Relatively higher prevalence of *Salmonella* in white soft cheese was detected by [Elbagory et al. \(2015\)](#). Soft cheese contains high levels of moisture and pH, particularly the surface of cheese contains high moisture and pH facilitated during ripening process so that these are more exposed to microbial growth as compared to those of hard or semi-hard cheeses ([Robinson, 2002](#)). Other reasons of contamination of white soft cheese by *Salmonella* might be use of bad quality milk, improper heat treatment, unhygienic manufacture practice or use of contaminated brine.

A PCR targeting *invA* gene (target size: 244-bp) was done conferring rapid identification of *Salmonella* isolates as all *Salmonella* serovars have the invasion gene (*invA*) as a unique character ([Herrera-Leon et al., 2004](#)). [Lampel et al. \(2000\)](#) also reported that PCR with primers for *invA* gene was rapid, sensitive and specific for the detection of *Salmonella* in many samples. On the other hand, *invA* gene was widely recognized as the marker for rapid identification of *Salmonella* genus ([Malorny et al., 2003](#)) (**Figure 1**).

The serotyping results showed that there were 6 serovars. *S. enteritidis* (33.3%) was the most common serovar, followed by *S. typhimurium* (25.9%), *S. heidelberg* (14.8%), *S. infantis* (11.11%), *S. tsevie* (11.11%) and *S. haifa* (3.7%). Similar results were also reported by [Haley et al., \(2014\)](#) who reported that *S. enterica subsp. Enterica* was the common isolate. However, [Hassan et al. \(2000\)](#), [Jayarao et al. \(2006\)](#), [Zagare et al. \(2012\)](#), [Mohammed et al. \(2013\)](#) and [Elbagory et al. \(2015\)](#) reported that *S. typhimurium* was the most common one.

*S. enteritidis* and *S. typhimurium* were detected in milk and cheeses samples. These were the most important serotypes of salmonellosis causing gastroenteritis transmitted from animals to humans in most parts of the world ([Fashae et al., 2010](#); [Hendriksen et al., 2011](#)), which is often uncomplicated and does not need any treatment, However, this can be severe in the younger and older humans, and patients with weakened immunity. But, it is generally reported that the presence of any *Salmonella* isolate in a food should be considered as a potential hazard to human as a high percentage of human salmonellosis occurs through consumption of raw milk or dairy products manufactured with raw milk ([CDC, 2003](#)).

In this study, *S. typhimurium*, *S. enteritidis*, *S. haifa* and *S. infantis* were detected in the kareish cheese, while *S. typhimurium* was the most prominent one. This was agreed

with [Shen et al. \(2007\)](#) who reported that *S. typhimurium* was adapted to acid at pH 5.5 for 4 h. So, acid adaptation is an important survival mechanism enabling *Salmonella* spp. to persist in fermented dairy products, and possibly other acidic food products.

Only one white soft cheese sample contained *Salmonella* (**Table 1**), this might be occurred manufacture, and can survive for more than 60 days in cheese ([ICMSE, 2006](#)). However, this sample proved to be the most contaminated one of the all examined samples and yielded five types of *Salmonella* serovars (*S. typhimurium*, *S. enteritidis*, *S. tsevie*, *S. infantis* and *S. heidelberg*) (**Table 2**). Many factors determine the quality of cheese as quality of the clotted skim milk, method of the manufacture, time required to drainage the whey, quality of added salt and method of handling the finished cheese ([Abou-Donia, 2008](#)).

PCR assay was used for detection of *Salmonella* before using the serological identification, as reported by [Eid \(2010\)](#) who found that some isolates were detected by PCR which had been detected as negative in serological examination. Also, [Wang et al. \(2013\)](#) reported that PCR was a rapid and accurate method for the detection of *Salmonella*, so it should be used firstly, as we did.

## CONCLUSION

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Six different *Salmonella* serotypes are isolated from milk and cheese samples, which may act as a source of human infection. For the prevention of *Salmonella* contamination in milk and milk products, we may apply proper hygienic measures during milking and handling of milk after milking. Efficient cleaning of all utensils and equipment, effective training and education the farmers to improve awareness of milk borne zoonosis and risk factors can be followed. Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Points (HACCP) systems are suggestive to avoid *Salmonella* contamination.

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## CONFLICT OF INTEREST

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The authors declare that they have no conflict of interest.

## AUTHORS' CONTRIBUTION

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AHEB designed the study, interpreted the data, and drafted the manuscript. MESES was involved in

collection of data and also contributed in manuscript preparation. AAK and MAAA took part in preparing and critical checking of this manuscript.

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