Original Research Article

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Prevalence of *Salmonella* species in milk and milk products in New Valley governorate

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ABSTRACT: Milk and its derivatives serve as significant reservoirs of *Salmonella*, particularly for individuals who favor the consumption of untreated milk. The prevalence of *Salmonella* infections is influenced by various factors, including unsatisfactory hygiene practices on farms, the conduct of food handlers, and the consumption of raw milk and milk-based products. *Salmonella* is a leading cause of salmonellosis, a prominent global foodborne illness. This study aimed to investigate the presence of *Salmonella* in marketable milk and some dairy products in New Valley Governorate, Egypt. A total of 150 random samples of commercially available milk and some milk products, such as soft cheeses (Kareish, Domiati), and ice cream (30 samples for each category), were collected from diverse locations within New Valley Governorate. The prevalence rates were found to be 1.6% for marketable milk, on the other hand, *Salmonella* species couldn't be detected in Kareish cheese, Domiati cheese, and ice cream samples. In conclusion, the detection of *Salmonella* in milk suggests that the examined samples exhibited suboptimal quality, indicative of unhygienic practices throughout the entire milking, processing, storage, and distribution processes.

KEYWORDS: *Salmonella*, milk, Kareish cheese, Domiati cheese, ice cream

1. Introduction

Food borne illnesses caused by pathogens constitute a significant global public health concern, with countries allocating substantial resources to combat this issue. Bacterial food infections are a source of apprehension for both developed and developing nations [1]. Ensuring the safety of food, particularly products of animal origin, has become increasingly crucial for human health. The rise in the consumption of animal products is accompanied by a heightened risk of raw food, has drawn attention to the consumption of raw milk as a commonly distributed raw product [2]. Milk, being a common element in the human diet, is easily accessible for immediate consumption. Nevertheless, it acts as a potential carrier for several pathogens, such as *Salmonella*, Staphylococcus aureus, and Campylobacter species [3]. Dairy products are susceptible to contamination with diverse microorganisms from various sources during production, processing, and handling, rendering them unfit for consumption, such as in the case of cheeses and ice cream [4]. Milk and its derivatives emerge as significant sources of *Salmonella*, particularly for consumers who prefer raw milk. Factors such as inadequate hygiene on farms, improper handling by food personnel, and the consumption of raw milk and milk products contribute to *Salmonella* infections [5]. *Salmonella* is a major cause of worldwide foodborne diseases known as salmonellosis [6]. Salmonellosis, a gastrointestinal food illness, results from numerous *Salmonella* serotypes, exceeding 2400 in number. Typical symptoms of *Salmonella* infection include acute gastroenteritis with sudden abdominal pain, diarrhea, nausea, vomiting, and, in severe cases, dehydration, particularly among infants. Fever is a consistent symptom, with anorexia and diarrhea persisting for several days. Over the last ten years, salmonellosis has become the second most commonly reported zoonotic disease and the primary cause of foodborne outbreaks in the European Union. In 2020, the notification rate for salmonellosis was recorded at 13.7 confirmed cases per 100,000 inhabitants, amounting to a total of 52,702 cases in humans throughout the European Union [7]. In some instances, the clinical progression may manifest as enteric fever or

septicemia, with or without focal infection, or initial gastroenteritis evolving into enteric fever or septicemia [8]. Therefore, this study aimed to examine the presence of *Salmonella* spp. in milk and chosen milk products.

2. Material and Methods

2.1. Collection and preparation of samples:

A total of 150 samples, chosen randomly, were collected from different sources within the New Valley governorate. These included marketable milk from both dairy farms and shops (30 samples each), various milk products like soft cheeses [5] from grocery stores (30 samples each), and ice cream from street vendors and small-scale producers (15 samples each). The samples were acquired in clean, dry, and sterile containers. After collection, they were promptly transported to the laboratory for analysis, following the procedures outlined by [9].

2.2. Isolation of Salmonella spp.[10]

The samples underwent examination to detect the presence of *Salmonella* spp. In this process, 25 ml or gm of each sample was taken and transferred to 225 ml of sterile buffered peptone water, followed by incubation at 37° for 24 h. Subsequently, 0.1 ml of the cultured broth from buffered peptone water was aseptically inoculated into 10 ml of selenite cysteine broth (CM0699), with the culture being incubated at 41° for 24 h. A loopful of the cultured selenite cysteine broth was then streaked onto Xylose-Lysine-Desoxycolate (XLD) (CM0469), and the mixture was incubated at 37° for 18-24 h. Suspected colonies were identified as pink or red colonies with or without black centers, as described by[11]. Isolates were subsequently confirmed using Triple Sugar Iron agar (TSI), Urease test, and Simmons citrate agar.

2.3. Polymerase chain reaction (PCR):

Purification Kit (ThermoFisher, Cat. No. K0702). PCR was employed to amplify the virulence gene invA of *Salmonella*. The forward primer sequence was 5' TATCGCCACGTTCGGCAA '3, while the reverse sequence was 5' TCGCACCGTCAAAGGAACC '3 [12]. The PCR reaction mixture (25 µL) included 3 µl of bacterial DNA, 12.5 µL of 10x Master Mix Green Master (Promega, USA). Additionally, 1 µL of each primer (Applied Biosystem, USA) and the reaction was completed by the addition of deionized water. The amplification process was carried out in a Gradient Thermal Cycler (Veriti Applied Biosystem, USA). The PCR cycling protocol comprised an initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 45 sec, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min. A final extension at 72 °C for 7 min concluded the process. Subsequently, 5µL of each amplicon underwent electrophoresis in a 1.5 % agarose gel, stained with ethidium bromide, and visualized and captured on a UV transilluminator (UV, INC, UK). A 100 bp DNA ladder served as a marker for PCR products. The amplified product size of invA gene was 275 bp.

3. Results

Table 1: Prevalence of *Salmonella* in the examined marketable milk samples.

Examined sample	No. of examined sample	Positive	
		No.	%
Dairy farm	30	1	3.3
Dairy shop	30	-	0
Total	60	1	1.6

Table 2: Prevalence of *Salmonella* in the examined marketable milk and some milk products samples.

Examined sample	No. of examined sample	Positive	
		No.	%
Marketable milk	60	1	1.6
Soft cheese	60	-	0
Ice cream	30	-	0
Total	150	1	0.6

4. Discussion

Salmonella infections, recognized globally, are categorized as foodborne illnesses, primarily transmitted

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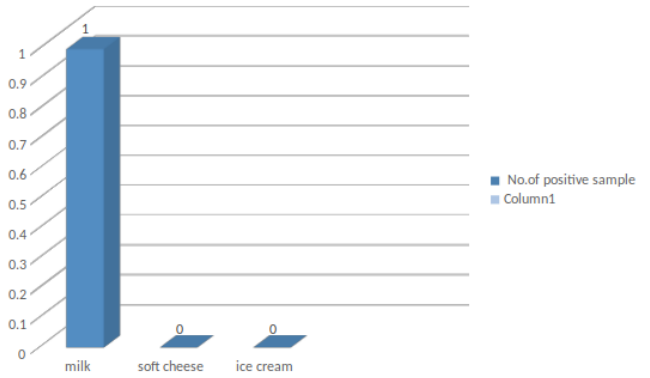
Figure 1: Prevalence of *Salmonella* in the examined marketable milk and some milk products samples.



through contaminated food. Outbreaks typically stem from food contamination at its source, occasionally occurring during handling by an infected individual or carrier and may even result from person-to-person transmission. Recent instances of *Salmonella* outbreaks linked to dairy products, particularly milk, have spurred global scientific investigations to assess the extent of *Salmonella* contamination in the dairy sector, potentially leading to the contamination of various dairy products [8]. Examining the outcomes presented in Table 1, it was observed that the overall prevalence of *Salmonella* species was 1.6% in the analyzed marketable milk samples. In dairy farm milk samples, the incidence of *Salmonella* was 3.3%. These findings regarding marketable milk samples align with those reported by [13]. However, other studies have reported higher incidences of the organism in examined marketable milk samples, as noted by [14, 8, 15, 16, 17, 18, 19]. Analyzing the data in Table 2, *Salmonella* was not detected in soft cheese. The absence of *Salmonella* in soft cheese may be attributed to its pH values (4.5-4.6), creating an unfavorable environment for *Salmonella* contamination during and after manufacturing. Conversely, some researchers suggest that casein provides protective effects against *Salmonella* in acid dairy products. While soft cheeses can be consumed immediately after production, posing potential public health risks, it is advisable to manufacture cheese solely from pasteurized milk under hygienic conditions to mitigate potential public health concerns related to *Salmonella*. Based on the information provided in Table 2, *Salmonella* was not identified in the ice cream samples. The lack of detection of *Salmonella* in ice cream could be attributed to reducing temperatures during the manufacturing and storage of ice cream is an effective measure to minimize the chances of *Salmonella* and other bacteria growth. Low temperatures act as an inhibitory factor for bacterial growth, thereby reducing the risk of bacterial contamination in the product. Low temperatures are typically employed during various stages of ice cream production, such as storing ingredients and preparing the mixture, and later during freezing This contributes to slowing down bacterial growth and preserving the quality and safety of the product. The potential connection between the presence of *Salmonella* in ice cream and its occurrence could be attributed to the utilization of contaminated water and other ingredients during the preparation process. This presents a food poisoning risk, particularly if the milk used in the production is tainted with *Salmonella*. The detection of *Salmonella* in ice cream indicates substandard quality and questionable safety practices during the manufacturing process. This includes the use of inferior ingredients such as fluids and dry components, as well as the addition of flavors, coloring agents, fruits, nuts, and chocolate chips to the mixture. Furthermore, the presence of poorly cleaned equipment, air incorporation, product rerun, and personnel can be identified as potential sources of post-pasteurization contamination [8]. Many virulence genes have been the causative of *Salmonella* pathogenicity, and the severity of infection depends mainly on the presence or absence of these genes. One of these virulence genes is invA gene is a biomarker for non-typhoidal *Salmonella* , which plays an important role in the invasion of the host epithelial cells [20].

Conclusion

The current investigation gathered fresh data concerning *Salmonella*'s presence in marketable milk and milk products within the New Valley governorate. Salmonellosis stands as a prevalent foodborne illness in humans, with documented outbreaks associated with the consumption of milk and its derivatives. Therefore, it is advised to utilize raw milk of superior quality for both direct consumption and the production of various items. Additionally, the application of pasteurization is strongly recommended for all raw milk used in the manufacturing processes of soft cheese, ice cream, or any other dairy products.

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