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Occurrence of non-typhoidal Salmonella in New Valley and Assiut provinces

Sotohy, Ahmed Sotohy [1], Asmaa, S Thabet [2], Mohamed Abd Elsalam [2], and Mohamed, S Diab [3]

[1]Department of Animal, poultry and environmental Hygiene, Faculty of Veterinary medicine, Assiut University
[2]Animal Health research institute, ARC, Assiut lab., Egypt.
[3]Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, New Valley University, El-Kharga, Egypt
*Corresponding author: zarzar_vet@yahoo.com
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ABSTRACT: The purpose of this study was to investigate the occurrence of non-typhoidal *Salmonella* (NTS) and the distributed serotypes in New Valley and Assiut provinces. Eight hundred samples were collected (280 from New Valley and 520 from Assiut) from different sources including fecal swabs (160), raw milk (80), Damietta cheese (80), Kareish cheese (80), ice cream (80), yoghurt (80), stool samples (170) and hand swabs (70). Samples were bacteriologically examined then the recovered isolates were serotyped. In New Valley province, occurrence of NTS was 8.57%, 3.33%, 0.00%, 20,00%, 17.14% and 5.71% of milk, Kareish cheese, Damietta cheese, yoghurt, fecal swabs and stool samples, respectively, while, the occurrence of NTS in Assiut was 15.56%, 18.00%, 12.50%, 4.44, 8.75%, 15.56%, 11.43% and 8.00% of milk, Kareish cheese, Damietta cheese, yoghurt, ice cream, fecal swabs, hand swabs and stool samples, respectively. 13 different serotypes were detected in the recovered isolates (87) including *S. Typhymurium* and *S. Enteritidis* were the predominant serotypes with an occurrence rate of 24.14% and 20.69%, respectively, followed by *S. Tsieve* (12.64%), *S. Infantis* (8.05%), *S. Larochelle* (9.20%), *S. Virchow* (5.75%), *S. Molade* (8.05%), *S. Haifa* (3.45%), *S. Shubra* (1.15%), *S. Alfort* (1.15%), *S. Essen* (2.30%), *S. Apeyeme* (1.15%) and S. Heidelberg (2.30%). In conclusion, the high prevalence of the NTS in various dairy products represents an alarm for the public health because of the possibility of transmitting these serovars to consumers, which requires reviewing the methods of control and prevention.
KEYWORDS: Non-typhoidal *Salmonella*, cattle, milk and milk products, human, serotyping.

1. introduction

Salmonella infections, both typhoidal and non-typhoidal, are widespread in Egypt and cause significant economic losses [1]. NTS are *Salmonella* species other than *Salmonella Typhi* and *Salmonella Paratyphi* is ranked second in its contribution to domestically acquired foodborne illnesses. The gastroenteritis caused by NTS species are estimated at about 93.8 million cases annually, counting about 35% hospitalization and 28% mortality of the infected cases. The highest mortality rates from *Salmonella* infections are primarily in the poorest developing world [2]. NTS usually causes self-limiting gastroenteritis with symptoms appear with in 12 to 72 hours after infection including diarrhea, abdominal pain and vomiting which may last for 4-7 days. However, in children, elderly and immunocompromised patients, NTS may cause severe gastroenteritis which need hospitalization [3, 4] . Recovered patients may carry NTS for about 3 to 7 days up to 7 weeks [5]. People generally acquire the pathogen through foodborne sources, direct contact with infected animals and person to person transmission[6]. NTS in animals are often carried asymptomatically and disease occurs usually when animals are placed under stress. Animals which had recovered from infection may continue shedding the bacteria for 2-12 weeks [7, 8]. Globally, around 86% of salmonellosis was of food borne sources [9]. Raw or improperly pasteurized Milk and their products are considered potential sources of *Salmonella* infection to humans [10]. Feces of infected cattle, contaminated skin, infected udder, milking equipment, air (dust borne infection), feed, animal insects and milkers are main sources of raw milk contamination with *Salmonella* [11]. From more than 2600 identified *Salmonella* serotypes, *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* have a major public health concern [12]. In view of the public health importance of NTS and its pandemic nature all over the world, it is important to study the prevalence of *Salmonella* and distributed serotypes in

Egypt. So, in this study, we investigate the occurrence of NTS in milk, milk products, cattle and human and the different serotypes in New Valley and Assiut provinces.

2. Materials and methods

2.1. Sample collection:

A total of 800 samples of fecal swabs (160), raw milk (80), kareish cheese (80), Damietta cheese (80), yoghurt (80), ice cream (80), stool samples (170) and hand swabs (70) were collected during the period of April 2020 to May 2021 from different localities in New Valley and Assiut provinces, Egypt. Samples were collected in sterile and sealed containers, labeled and placed in ice box at 4ºC and transported to the laboratory for microbiological analysis.

2.2. Isolation and identification of NTS:

NTS was detected using conventional culture-based methods as reviewed in ISO [13]. Each sample was pre-enriched in buffered peptone water then incubated for 24 hours at 37°C. 0.1 ml of the pre-enriched broth was enriched in 10 ml of Rappaport Vassilliadis (RV) broth then overnight incubation at 42°C. A loopful from selective enrichment broth was inoculated on XLD agar (Xylose Lysine Desoxycholate) and incubated for 24 hours at 37°C. Pink to red colonies with or without black center were picked and streaked on nutrient agar slopes and incubated for 18-24 hours at 37°C for biochemical identification according to [14] by using indole production test, Simmons citrate test, urease test, triple sugar iron and sugar fermentation test then confirmed with API (Analytical profile index).

2.3. Serotyping:

Serological identification of *Salmonella* isolates according to Kauffman-White scheme [15] were carried out at the Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Benha University, Egypt by the slide agglutination technique of both somatic (O) and flagellar (H) antigens.

2.4. Ethical approval:

The ethical approval was received from Faculty of medicine, Assiut University, Egypt (04-2023-100084).

3. Results and Discussion

Table 1: Overall occurrence of NTS in samples of animal origin

Type of sample	New Valley			Assiut			Total		
	No.	Positive	%	No.	Positive	%	No.	Positive	%
Milk	35	3	8.57	45	7	15.56	80	10	12.50
Kareish cheese	30	1	3.33	50	9	18.00	80	10	12.50
Damietta cheese	40	0	0	40	5	12.50	80	5	6.25
Yoghurt	35	7	20.00	45	2	4.44	80	9	11.25
Ice cream	-	-	-	80	7	8.75	80	7	8.75
fecal swabs	70	12	17.14	90	14	15.56	160	26	16.25
Total	210	23	10.90	350	44	12.60	560	67	11.90

Salmonellosis is a zoonotic infection that causes substantial economic losses resulting from morbidity, mortality and poor growth with hazard of transmitting food poisoning to human. Based on conventional methods and serotyping, 87 NTS isolates were isolated from 800 samples collected from New Valley and Assiut including milk, milk products, fecal swabs, Hand swabs and stool samples with occurrence rate of 10.88% including 11.90% of samples of animal origin and 8.30% of samples of human origin. Occurrence of NTS in samples of animal origin was higher in Assiut (12.60%) than in New Valley (10.90%) (Table 1). Moreover, occurrence of NTS in samples of human origin was slightly higher in Assiut (11.60%) than in New Valley (11.40%) (Table 2). These results was in agreement with [16] who found insignificant differences in *S. Typhimurium* prevalence among selected districts in their study. In another study of [17], they found high level of statistical significance in the prevalence of *Salmonella* in different districts. [18] reviewed that there was a great variation in the prevalence of NTS from region to region, even within the same country, which is depending on the climatic conditions, hygienic measures, management practices of farm, handling, processing and storage of raw food. It may be also affected by differences in sample type, sampling season, sampling methods and the employed isolation techniques. Milk and milk products are considered major sources of *Salmonella*. Improper hygienic conditions in the farm, food handlers and consumption of raw milk and milk products are main sources of *Salmonella*

Table 2: Occurrence of NTS in samples of human origin

Type of sample	New Valley			Assiut			Total		
	No.	Positive	%	No.	Positive	%	No.	Positive	%
Hand swabs	-	-	-	70	8	11.43	70	8	11.43
Human stool	70	4	5.71	100	8	8.00	170	12	7.06
Total	140	16	11.40	190	22	11.60	240	20	8.30

Table 3: Occurrence of NTS in animals in relation to healthy status

Result of samples examination	Healthy cattle (n= 89)		Diarrheic cattle (n= 71)	
	No.	%	No.	%
Positive	13	14.61	13	18.31

Table 4: Occurrence of NTS in human in relation to contact with animals

Samples	Contact human (n= 158)		Non-contact human (n= 82)	
	No.	%	No.	%
Positive	15	9.49	5	6.10

infections [17, 19]. Results in (Table 1) showed that NTS was detected in 12.50% of the examined raw milk samples collected from dairy shops and dairy farms in New Valley and Assiut provinces. The occurrence of NTS in raw milk was higher in Assiut (15.56%) than in New Valley (8.57%). Occurrence of NTS in raw milk in this study was higher than the occurrence reported by [20] who investigated that 6.66% of each market raw milk and bulk tank milk was contaminated by NTS. Also, lower occurrence was observed by [21] who isolated NTS from 3% (4 out of 133) of raw market milk samples but they failed to isolate *Salmonella* from milk samples collected directly from teat of lactating cows. On the other hand, [22] estimated higher occurrence (20.5%) of NTS in raw milk collected directly from the mammary gland during milking than in our study. Omar, et al. [23] illustrated that high presence of *Salmonella* among raw milk may be attributed to shedding of *Salmonella* into milk from the infected udder or contamination of raw milk with feces during its collection. Kareish cheese is one of the most popular cheeses consumed in Egypt due to its high protein, low fat and affordable price. Data illustrated in (Table 1) reveals that NTS was isolated from totally 80 Kareish cheese samples with a percentage of 12.50%. NTS was isolated from 3.33% and 18% of Kareish cheese samples collected from

Table 5: occurrence of NTS in human in relation to healthy status

Positive samples	Healthy human (n= 84)		Diarrheic human (n= 86)	
	No.	%	No.	%
	2	2.38	10	11.63

New Valley and Assiut, respectively. Lower occurrence of NTS in New Valley may be due to high salting of kariesh cheese collected from New Valley. Variable occurrences of NTS in Kareish cheese was reported in many previous literatures. [20] found high NTS prevalence rate at 16.67% in the examined kareish cheese samples. In another study, [24] added that the contamination of kareish cheese with NTS was high (20%) in their study due to improper sanitation practices during manufacture and handling of cheese and most cheeses were sold uncovered or without container. On the other hand, [23] recorded a lower prevalence (8%, 2 out of 25) of NTS obtained from Kareish cheese than our finding. The illustrated results in (Table 1) revealed that 6.25% of the examined Damietta cheese were contaminated by NTS. All positive stains (12.50%, 5 out of 40) were isolated from samples collected from Assiut while, NST couldn't be isolated from Damietta cheese samples collected from New Valley. Lower occurrence of NTS in Damietta cheese was found by [25] who could isolate NTS from 4% of the examined Damietta cheese samples. However, higher occurrence was reported by [26] who isolated NTS from 20% (10 out of 50) of the examined Damietta cheese. [24] could isolate NTS from 4% (1 out of 25) of white soft cheese. They illustrated that there are high levels of moisture and pH in soft cheese, particularly the surface which facilitated during ripening process so that soft cheeses are more exposed to microbial growth than other hard or semi-hard cheeses. They added that the other causes of soft cheese contamination with *Salmonella* are bad quality milk, improper heat treated milk, unhygienic manufacture practices or use of contaminated brine. Yoghurt is the most popular milk product that constitutes a main part of both child and adult diet. Results showed in (Table 1) revealed that NTS was isolated from 11.25% (9 out of 80) of the examined yoghurt samples in this study. From these 9 positive isolates, 7 isolates (20%) were from New Valley and 2 isolates (4.44%) were from Assiut. Our result was different from those found by other researchers. [27] isolated NTS with higher percentage of 24% from yogurt while, [28] failed to isolate

Table 6: Serotypes of NTS isolated from different samples

Type of samples	Milk (n=80)		Kareish cheese (n=80)		Damietta cheese (n=80)		Yoghurt (n=80)		Ice-cream (n=80)		Animal fecal swabs (n=160)		Hand swabs (n=70)		Human Stool samples (n=170)		Total (n=800)	
Serotypes	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. Typhimurium	3	3.75	4	5.00	0	0	2	2.50	0	0	8	5.00	3	4.29	1	0.059	21	2.63
S. Enteritidis	2	2.50	0	0	1	1.25	2	2.50	3	3.75	5	3.13	1	1.43	4	2.35	18	2.25
S. Tsieve	3	3.75	0	0	1	1.25	1	1.25	1	1.25	2	1.25	0	0	3	1.76	11	1.38
S. Infantis	0	0	2	2.50	1	1.25	1	1.25	1	1.25	1	0.63	1	1.43	0	0	7	0.88
S. Larochelle	1	1.25	0	0	0	0	0	0	0	0	4	2.50	2	2.86	1	0.059	8	1.00
S. Virchow	0	0	2	2.50	0	0	0	0	0	0	3	1.88	0	0	0	0	5	0.63
S. Molade	0	0	0	0	0	0	1	1.25	2	2.50	1	0.63	1	1.43	2	1.18	7	0.88
S. Haifa	1	1.25	1	1.25	0	0	0	0	0	0	1	0.63	0	0	0	0	3	0.38
S. Shubra	0	0	0	0	1	1.25	0	0	0	0	0	0	0	0	0	0	1	0.13
S. Alfort	0	0	0	0	1	1.25	0	0	0	0	0	0	0	0	0	0	1	0.13
S. Essen	0	0	0	0	0	0	1	1.25	0	0	1	0.63	0	0	0	0	2	0.25
S. Apeyeme	0	0	1	1.25	0	0	0	0	0	0	0	0	0	0	0	0	1	0.13
S. Heidelberg	0	0	0	0	0	0	1	1.25	0	0	0	0	0	0	1	0.059	2	0.25
Total	10	12.50	10	12.50	5	6.25	9	11.25	7	8.75	26	16.25	8	11.43	12	7.06	87	10.88

Table 7: Occurrence of different serotypes of NTS in the recovered isolates

Serotype	Positive	%
S. Typhimurium	21	24.14
S. Enteritidis	18	20.69
S. Tsieve	11	12.64
S. Infantis	7	8.05
S. Larochelle	8	9.20
S. Virchow	5	5.75
S. Molade	7	8.05
S. Haifa	3	3.45
S. Shubra	1	1.15
S. Alfort	1	1.15
S. Essen	2	2.30
S. Apeyeme	1	1.15
S. Heidelberg	2	2.30
Total	87	100

Salmonella from any of yoghurt samples analyzed. The difference in hygienic practices during preparation of yoghurt may be resulted in this variation. The illustrated results in (Table 1) showed that 8.75% (7 out of 80) of ice cream samples collected from Assiut were confirmed to be NTS positive. Unexpected higher occurrence obtained by [23] who investigated 72% of the examined ice cream to be NTS positive. They added that the unexpected high occurrence of NTS in ice cream samples may be resulting from repeat freezing and thawing of ice cream due to loss of electricity especially in developing countries in addition to the post processing contamination especially in small scale manufacture. Similarly, [29] found that *Salmonella* was detected in 68.8% of ice cream samples and the high occurrence was due to using of raw milk, unhygienic measures and improper washing of dishes. On the other side. [30] failed to isolate *Salmonella* from 144 ice cream samples were collected from Urmia City, Iran. [31] explained that the variation in occurrence of *Salmonella* in milk and its products could be attributed to source of samples, sampling techniques, seasonal variation, geographical differences, methods of isolation and process of product manufacturing. *Salmonella* infection in cattle farms is causing economic losses and hazard of transmission to human so it is continuing to be a major public problem. In the present study, the overall occurrence of NTS in cattle was 16.25% out of 160 fecal swabs collected from different farms of New Valley and Assiut provinces (Table 1). The occurrence of NTS in cattle was slightly higher in New Valley (17.14) than in Assiut (15.56%). The obtained rate of NTS was higher than that reported by [18] who isolated NTS from 5% (5 out of 100) of the examined fecal swabs collected from cattle. They added that there is a great variation in the prevalence of NTS in livestock from region to region, even within the same

country, which is depending on the climatic conditions, hygiene and management practices in farms. It may be also affected by differences in sampling season, sample type, sampling methods and isolation techniques. According to data in (Table 3), NTS could be isolated from 14.61% and 18.31% of apparently healthy cattle and diarrheic cattle, respectively. These findings were in agreement with [32] who reported the highest percentage of *Salmonella* isolates from diarrhetic animals (9.4%) compared to only 2.1% from healthy animals and this variation was statistically significant. In another study was conducted by [33], *S. Enteritidis* was isolated from 0.8% (2/250) of healthy cows while, it could not be isolated from diarrheic cows. On the contrary, 1.3% (2/150) of diarrheic buffaloes was positive for S. Typhimurium and none of the healthy buffaloes carried *Salmonella*. They added that these results were not statistically significant. Unexpected high occurrence of NTS in healthy cattle reflects the role of carrier animals in the spread of infection so, hygienic measures must be taken even during contact with apparently healthy animals and periodical examination of animals is very important. Human salmonellosis represents a public health problem in both developed and developing countries. It is one of the most common causes of foodborne infection in human beings and still the main cause of acute diarrhea[34, 35, 36, 37]. From data in (Table 2), we found that the occurrence of NTS in stool samples collected from human was 7.06%. The percentage of NTS in human stool was higher in Assiut (8.00%) than in New Valley (5.71%). The obtained results was higher than results detected by [34] who estimated the overall prevalence of NTS among human to be 4.4% and [38] who reported 5% prevalence of NTS in human. In this study, 70 hand swabs were collected from handlers of milk and milk products and farm workers in Assiut for detection of NTS. We found that 11.43% of these samples were positive for NTS (Table 2). The occurrence of NTS in hand swabs in this study was higher than other studies in which [39] isolated *Salmonella* from 3.7% (1 out of 20) of the examined hand swabs collected from some dairy workers while, [36] could not isolate *Salmonella* from 20 hand swabs collected from milkers, hands. [32] explained that contaminated dairy products and contact with dairy cattle represent a common source of NTS in humans. In our study we observed that the occurrence of NTS was higher in human who was in contact with animals and animal products (milk and milk products, handlers and farm workers) (9.49%) than non-contact human (6.10%) (Table 4) Higher percentage of NTS in contact human than its percentage in non-contact human with the observed high occurrence of NTS in hand swabs highlights the role of food handlers and farm workers as a source of transmission throughout the chain of production, processing, storage and preparation. The mishandling of food and the disregard of hygienic measures enable pathogens to come into contact with food and in some cases, to survive and multiply in sufficient numbers to cause illness in consumers [40]. As illustrated in (Table 5), NTS was isolated from healthy human at a percentage of 2.38% while, the percentage of NTS from diarrheic human was higher (11.63%). Isolation of NTS from healthy human clarifies the role of carriers in transmission of infection. A variable findings was obtained by [41] who reported 3.75% prevalence of NTS (42 out of 1121) from diarrheal patients and 0.31% (1 out of 319) from non-diarrheal patients. Another study was performed by [42] in which they found that six strains of *Salmonella* were isolated from stool samples collected from diarrheal patients with an incidence of 3.75%. A higher occurrence of NTS in diarrheal patients was reported by [43] who examined a total of 255 fecal specimens and found that 20.39% (52 out of 255) were positive for NTS. Thirteen different stereotypes were identified in the recovered isolates (87) with difference in their distribution in different sample types as declared in (Table 6). *S. Typhymurium* and *S. Enteritidis* were the predominant serotypes with an occurrence rate of 24.14% and 20.69%, respectively, followed by *S. Tsieve* (12.64%), *S. Infantis* (8.05%), *S. Larochelle* (9.20%), *S. Virchow* (5.75%), *S. Molade* (8.05%), *S. Haifa* (3.45%), *S. Shubra* (1.15%), *S. Alfort* (1.15%), *S. Essen* (2.30%), *S. Apeyeme*

(1.15%) and S. Heidelberg (2.30%) (Table 7). Nearly, our results agree with findings of [44, 45], who mentioned that the most prevalent serotypes in their studies were *S. Typhymurium* and *S. Enteritidis*. Different findings were recorded by [46] who demonstrated that the main serovars in their study were *S. Dublin* (n=10, 35.7%) and *S. Virchow* (n=5, 17.9%) followed by S. Braendrerup, *S. Haifa* and *S. Saintpaul* which were isolated from 2 samples each (7.1%). Another study was performed by [22] in which the most notable *Salmonella* serovars isolated were S. Newport (60.87%), *S. Typhimurium* (17.4%), *S. Virchow*, *S. Bredeney* (4.3%) and *S. Anatum* (4.3%).

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