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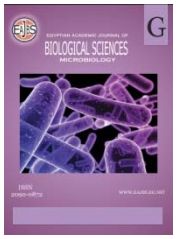

Detection and Growth of *Eschericia coli* and *Salmonella* in Jarjeer/Rocca while in Transit and Storage and Their Presence in the Prepared Salad Mixture Called Tabouleh

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ABSTRACT

Bacterial analysis of jarjeer, five years after an earlier study, verified that the level of contamination of salad greens by coliforms (14,644,283 cfu/g) and *E. coli* (2,749,906 cfu/g) was statistically significantly higher(P= 0.026) and occurring in 100% of the samples. Additional tests for *Salmonella* confirmed that this pathogen was also present on the surface of the leaves as well as sequestered inside the leaf cells. Jarjeer had 57,606 cfu/g *E. coli* and 61,277 cfu/g *Salmonella*, while parsley had 60,500 cfu/g *E. coli* and 75,750 cfu/g *Salmonella*. *Salmonella* and *E. coli* tests of the salad mixture tabouleh also showed extensive amounts for both of these bacteria and confirmed their abundant presence in the prepared salads. The leaf surface *Salmonella* and *E. coli* were reduced with a mild disinfectant wash, but these sequestered bacteria still remain alive inside the leaf tissues. Jarjeer leaves were incubated between 25°C and 40°C and the total coliform numbers increased significantly over a 24-hour period at temperatures ideal for coliform growth. The greatest increase occurred at 35°-40°C, which is the optimum temperature for *Salmonella*. Total coliforms and *E. coli* are multiplying inside the jarjeer leaves during shipment and storage, especially near the temperature range ideal for human pathogens.

INTRODUCTION

Popular salad items grown in the United Arab Emirates (UAE) include *Eruca sativa* ("jarjeer" or "rocca" mustard greens), *Petroselinum crispum* (parsley), *Mentha spicata* (Mint), *Allium cepa* (spring onions), *Coriandrum sativum* (coriander), *Latuca sativa* (lettuce), and other items that are normally eaten without cooking. These greens, particularly jarjeer, were tested for total coliforms and *E. coli* and were found to be highly contaminated with these bacteria(Russell *et al.*, 2010). The contamination was not only superficial, but persisted even after several washings and disinfecting with 5% bleach. The data presented showed that fecal coliforms plus *E. coli* were sequestered within the epidermis cells of the jarjeer leaves and could not be washed off.

The numbers of *E. coli* released per gram of jarjeer greens were (42,059 – 833,812 cfu/g) and most probably represented a persistent health threat for those persons eating these fresh salad greens. Salad greens are one of the most likely means by which people are infected by enteric pathogens (Davis and Kendal, 2007) and the presence of *E. coli* indicates other, less easily detectible viral and bacterial pathogens are most probably also present.

The research done by Russell *et al.*, (2010) tested for the presence of total coliforms, and *E. coli* in particular, but did not test for the presence of *Salmonella* or *E. coli*0157:H7. Total coliforms represent the number of bacteria present from several species of coliform bacteria growing in the gut, but often the exact species are not known. However, it is relatively easy to detect and identify *E. coli* compared to *Salmonella*. It is well known that many strains of *E. coli* are harmless and beneficial to the intestinal flora, but the presence of coliforms in general and especially *E. coli* indicates the food or produce being tested is contaminated by feces and there could be other, less easily detected pathogenic bacteria, such as *Salmonella*, present in the food.

Salmonella species are associated with food borne diseases in every country and there are about six species and many strains or serotypes (Cetinkaya *et al.,* 2008). *Salmonella* is found naturally in the feces of farm animals, birds, reptiles, turtles, other animals, and sometimes humans. Symptoms of salmonellosis begin about 12 hours after ingesting *Salmonella* contaminated food and include diarrhea, enteric fever, gastroenteritis, abdominal cramps, and dehydration (Venes 2013; www. foodborneillness. com). Some people do not develop symptoms and if they do become sick most recover, but a few victims need to be hospitalized due to severe dehydration. The very young and the elderly are most prone to serious infections, which can become fatal if the bacteria enter the bloodstream (bacteremia) via perforation of the intestines (Pui *et al.,* 2011; www.cdc.gov). In 2015, the FDA recalled more than 50 different food items in the United States due to *Salmonella* contamination (www.fda.gov). Even trace amounts of *Salmonella* in food is considered dangerous to the public's health because the infectious dose is as low as 10 cells although the infectious dose range is very broad, from 1-10 billion cfu/g (Pui *et al.,* 2011).

The six objectives of this present research were:1) to confirm the previous investigation results by Russell *et al.,* 2010, and to verify that the numbers of total coliforms and *E. coli* continue to be present in jarjeer; 2) to extend the testing to include *Salmonella*; 3) to test for the presence of *E. coli* and *Salmonella* on the surface of certain produce leaves; 4) to test for the presence of *E. coli* and *Salmonella* in jarjeer/rocca, parsley, mint, and spring onions which are the main ingredients for the prepared salad mixture called tabouleh and to test tabouleh purchased from local grocery stores and restaurants; 5) to test the effectiveness of a commercial produce disinfectant; and 6) to test if bacteria are multiplying at different temperatures while the produce is in transit and storage.

MATERIALS AND METHODS

Testing Salad Greens for Coliforms and *E. coli* Using the IDEXX Method

The first test was used throughout the study and included jarjeer greens sold at local markets in five of the seven emirates. Jarjeer and other salad greens come as leaves attached to a small tap root and wrapped by a rubber band and placed on a stack that is not sprayed or refrigerated; parsley comes as leaves bundled in a similar manner. Jarjeer samples were taken randomly from stores in the seven emirates of the United Arab Emirates (UAE). Monitoring was done to see if there was any positive change or improvement in food quality over the past five years since the initial research reported by Russell *et al.,* (2010). Subsamples

weighing 10.5g were tested for total coliforms and *E. coli* using the IDEXX method (IDEXX Laboratories, Inc., Maine, USA; Clesceri *et al.,* 1999). Control samples of jarjeer were taken from a local hydroponics farm and also from a private garden.

Preliminary tests showed that maceration of washed vegetables for one minute in a sterile blender with 1 L sterile water was adequate to release the sequestered bacteria. A dilution series (1ml in 99ml; 0.1 ml in 99.9ml) from this water was done before testing with IDEXX and subsequent incubating of the samples for 24 hrs at 30-35^{o}C. These tests were done to broaden and verify the study done by Russell *et al.,* (2010) and to establish if the vegetables were now less contaminated nation-wide or not.

Testing for *Salmonella* Using Differential Agar

The second test extended the prior research beyond what had been done by Russell *et al.,* 2010, and to also detect the presence of *Salmonella* contamination of jarjeer and other greens. *Salmonella* chromogenic agar base with *Salmonella* selective supplement (Oxoid CM1007 and SR0194E) was used. Normally, a food sample needs pre enrichment with Rappaport broth to increase the numbers of *Salmonella* high enough for detection before further testing using agar plates (De Silva *et al.,* 2013), but preliminary tests showed that no enrichment was needed, because the numbers of *Salmonella* on jajeer and parsley were very high. Instead, a dilution series was required to reduce the numbers low enough to enable identification and so they could be counted .A 0.1ml water sample from the macerated vegetable was placed on agar plates using the standard spread plate technique. A broth cultures of cultured *Salmonella* and *E. coli* were used as positive controls. *Salmonella* chromogenic agar differentiates between *E. coli* and *Salmonella* colonies and can identify the presence of four common species of *Salmonella*.

Presence of *Salmonella* on the Surface of Cleaned Leaves

The third test was for the presence of *Salmonella* on the surface of cleaned jarjeer and parsley leaves. Leaf prints were made on *Salmonella* differential media agar plates. The leaves were washed twice in sterile water, air dried for a few minutes in a laminar flow hood, and then gently pressed onto the surface of the agar plates and incubated at 30-35^{o}C for 24hrs.

Tests for *E. coli* and *Salmonella* in Prepared and Purchased Tabouleh Salads

The fourth test was of tabouleh, a popular Arab salad prepared from mixture of chopped parsley leaves, mint, spring onions, tomatoes, olive oil, and lemon juice. The individual parsley leaves, the main component of tabouleh, were bought and tested separately. Tabouleh salads were also purchased randomly from different restaurants and grocery stores. The lemon water/oil mixture was squeezed out and tested using both the IDEXX and the *Salmonella* chromogenic agar techniques. A dilution of 0.1ml salad water into 99.9ml sterile water for IDEXX and 0.1ml of this dilution was also used for the Oxoid spread plate. The dilution reduced the numbers of *Salmonella* to a readable level.

Food Disinfection Technique

The fifth test was to see if the Food Saf Salad Wash, which is used by consumers, is an effective disinfection method for salad greens as the label states: "…kills all known harmful bacteria including *E. coli*, *Salmonella* and *Listeria*". According to the directions, one tablet was added to 5L sterile water and used to disinfect 10g of washed jarjeer for 10 minutes. After rinsing a second time, the jarjeer was tested using IDEXX and *Salmonella* selective agar.

Temperature Series Test for Growth of Coliforms, *E. coli* and *Salmonella* in Jarjeer Leaves during Storage and Transit.

The sixth test examined the growth of bacteria in jarjeer leaves during storage. Results from previous experiments (Russell

et al., 2010) showed there was more contamination of jajeer during the warmer (40°C) summer months than during the cooler (25°C) winter months. A temperature series was run to test if bacteria were multiplying in the leaves while in transit and storage. Four temperatures (25°, 30°, 35°, and 40°C) were used. A typical bundle of jarjeer (650g) was purchased from a local store and sub-divided into four lots of 100g each. These were placed, unwashed, into separate loosely closed plastic bags to reduce moisture loss and placed in separate incubators. Samples of 5g were removed from each bag at 4-6 hour intervals for 20 hours. They were tested with IDEXX and *Salmonella* selective agar.

Statistical Analysis

All numbers derived from the IDEXX method automatically include statistics that put the resulting data within the 95% confidence range (IDEXX Laboratories, Inc., Maine, USA; Clesceri *et al.,* 1999). Additional statistics (T-tests) were performed using Minitab16, Minitab Inc. State College according to Rao (1998).

RESULTS AND DISCUSSION

Coliforms and *E. coli* in Jarjeer Greens Remain at High Levels Five Years after the Initial Report.

Samples of jarjeer from each of the seven emirates five years after the original study done by Russell *et al.,* 2010, verified that the level of contamination of jarjeer greens by coliforms and *E. coli* remained high and was still occurring in 100% of the samples gathered from local stores. The average of washed jarjeer total coliforms was 14,644,283 ±16,863,983 cfu/g jajeer and the average for macerated after washing was 7,163,316 ±40,809,361 cfu/g. The average of washed jarjeer for *E. coli* was 2,749,906 ±2,972,212 cfu/g jarjeer. It is clear from the data that there was still a very large difference in levels of contamination between samples, some samples having very low contamination and others extremely high contamination (Table 1). In a few cases bacteria were well above the maximum (>241,960) for the capacity of the IDEXX test and the *E. coli*num bers ranged from 520 – 11,460 cfu/g (Table 1). The data shown in Table 1also contain numbers that look like they are higher (e.g. 48,820,000) than the maximum possible (e.g. >2,395,643) using IDEXX, but these higher values are due to having countable results from IDEXX that could be calculated back to real numbers of bacteria according to the dilution series used. This further illustrates that the results of sampling have erred on the low side of the contamination numbers, because the maximum number of >2,395,643 cfu/g actually means that the numbers are higher than ca 2,400,000 cfu/g plus other results shown in Table 1 suggest such numbers are at least 10X higher (*ca* 24,000,000 cfu/g).

When these data are compared to the Russell *et al.,* 2010 data it shows that there was no reduction of contamination indicated in the samples taken five years later. Results from earlier samples of jarjeer were (coliforms = 1,810,977 cfu/g; *E. coli* = 224,250 cfu/g) and present numbers are (coliforms = 14,644,283 cfu/g; *E. coli* = 297,221 cfu/g). All sample counts for bacteria taken from the different emirates remained high for jarjeer and the total coliform contamination had actually increased significantly (P = 0.026 confidence that the bacteria in 2015 were present in higher numbers than in 2010). When the jarjeer was washed and macerated the sample data showed that bacteria remain sequestered inside the leaves as before. *E. coli* remained at high levels even after being washed, as was shown by increased *E. coli* numbers after maceration (573,913 *E. coli* cfu/g) (Table 1).

There was no difference between contamination in the different samples from the five emirates. Initially it appeared that organically grown jarjeer was less contaminated, but upon maceration, these samples also resulted in equally high numbers of *E. coli*/g. The jarjeer samples which were organically certified were just as contaminated as those that were not organically certified (Table 1). Organically

grown jarjeer was contaminated with very high numbers of total coliforms (8,349,515 cfu/g) after being washed and macerated (Table 1). The basic ideal principle is that uncooked raw salad greens should not contain any coliforms, especially *E. coli* and *Salmonella*. The maximum safe Viable Bacteria Count (VBC), which includes all viable microorganisms, to consume in vegetables is 10^5 cfu/g. This is magnitudes lower than what was generally found in the present investigation for only the total coliforms not including all of the Viable Bacteria Count, which would logically be higher (De Silva *et al.,* 2013; Silva *et al.,* 2007).

Table 1: Jarjeer samples collected from five of the seven United Arab Emirates, AD (Abu Dhabi), AJ (Ajman), (DB) Dubai, RK (Ras Al Khaimah), and SH (Sharjah).

Treatment	Coliforms cfu/g	*E.coli* cfu/gStore	Emirate		Washed Averages by Emirate	
					$\bar{x}$Coli/g	$\bar{x}$*E.coli*/g
Washed	>2,395,643	10,891	CC	AD		
Macerd	>2,395,643	18,317	CC	AD		
Washed	48,820,000	1,710,000	SS	AD		
Macerd	34,800,000	290,900	SS	AD		
Washed	32,519,231	21,028,846	GG	AJ		
Macerd	2,326,154	2,326,154	GG	AJ		
Washed	222,547	0	LH	AJ	16,370,889	10,514,423
Macerd	2,282,262	712,736	LH	AJ		
Washed (co)	9,147,059	0	UC	DB		
Macerd (co)	8,349,515	291,262	UC	DB		
Washed	23,126,214	97,087	UC	DB	16,136,636	48,543
Macerd	9,401,961	294,118	UC	DB		
Washed	11,198,113	3,518,868	CC	RK	11,198,113	3,518,868
Macerd	2,282,264	1,226,132	CC	RK		
Washed	583,333	185,185	FG	SH		
Macerd	229,722	926	FG	SH		
Washed	3,786,408	573,914 ±769,652	SC	SH	2,184,870	384,214
Macerd	2,219,450	4,679	SC	SH		
Coliforms	Washed$\bar{x}$=	14,644,283±16,863,983				
Coliforms	Macerd$\bar{x}$=	7,163,316 ± 40,809,361				
E.coli	Washed$\bar{x}$=	2,749,906 ± 2,972,212				
E.coli	Macerd$\bar{x}$=	199,029	SC			

Coded store names, CC, GG, LH, SS, SC, UC. (co) is certified organic jarjeer. Jarjeer was tested after one wash. Macerd indicates the sample was macerated. Data are Most Probable Numbers (MPN) statistically valid to 95% confidence. Number of samples N=9.

Salmonella and *E. coli* were present on the surface of washed leaves

Salmonella is a more important concern than *E. coli* in salad greens. *Salmonella* presence in food is usually very low and samples most often samples need to be enriched with a broth culture before *Salmonella* can be detected. However, the salad greens tested here showed such high counts of *Salmonella* that the wash water samples needed to be diluted to reduce the numbers to enable accurate measurements and counts. Even after being diluted (1:99) there were so many colonies of *Salmonella* present on the surface of the agar from the leaves, a (+) (--) system had to be used in most cases instead of direct colony counts. When the colony counts on agar from washed leaves were low enough to read, jarjeer had 57,606 cfu/g *E. coli* and 61,277 cfu/g *Salmonella*, while parsley had 60,500 cfu/g *E. coli* and 75,750 cfu/g *Salmonella*.

In addition, washed and air surface dried fresh salad leaves were pressed onto indicator agar and resulted in directly transferring some of the bacteria to the agar surface. Most often the plates were over-run with *Salmonella* colonies. Jarjeer and

parsley both had abundant *Salmonella* and *E. coli* on their leaf surfaces. Most hydroponic and garden grown samples had no *Salmonella* or *E. coli*, but some had small amounts (1-2 colonies) of *Salmonella* (Table 2). Perhaps the contamination on hydroponic and garden samples came from dust containing wild bird feces. Disinfection of store purchased salad leaves reduced the amount of surface contamination, but did not reduce it to the very low levels that were seen in the control samples (hydroponic and garden grown greens) Table 2.

Table 2: Coliforms, *E. coli* and *Salmonella* present on the surface of washed jarjeer and parsley leaves..

Jarjeer samples	*Salmonella*	*E. coli*
Store 1	+++	+++
Store 2	++++	++++
Store 3	+++	+++
Hydroponic 1	+	0
Hydroponic 2	0	0
Hydroponic 3	0	+
Garden 1	0	0
Garden 2	0	+
Parsley samples		
Store 1	++	++
Store 2	++++	
Store 2	+++	+++

Colonies and amounts are given as: none = 0 colonies, + = low 1-10 colonies, ++ = moderate 10-100 colonies, +++ = high >100 colonies, and ++++ = very high >500 colonies

E. coli and *Salmonella* Are Present in Purchased Tabouleh Salads

The main ingredient of tabouleh is parsley and the fully prepared tabouleh salads sold in markets showed similar contamination, as was the expected result. The contamination of the ingredients (parsley, mint, and spring onion) used to prepare tabouleh was reflected in the contamination of the finished salad (Table 3). There was no difference between the contamination levels between tabouleh purchased from the four different major stores. The total coliforms and *E. coli* were high in the main ingredients (Table 3) and were also high in the finished product in every sample. Most notably were the *Salmonella* levels in tabouleh: all samples showed extensive *Salmonella* colony growth and thus the abundant presence of *Salmonella* in the salads. Even low contamination by *Salmonella* in any uncooked product offered for human consumption is unacceptable and cause for concern (Pui *et al.,* 2011).

Incidence of "food poisoning" in the UAE is about 550 cases a year, but over 1,120 suspected cases were reported and the actual number is probably higher (Masudi, 2014). Masudi (2014) reported that "the disease trigger could be anywhere along the food chain." *Salmonella* is a major cause of diarrheal diseases and any source of infection, such as is seen in the contaminated salad greens investigated here, can adversely affect public health.

Marginal Effectiveness of a Commercially Produced Disinfecting Product for Produce

Disinfection with a commercially available vegetable cleaning product resulted in a reduction of the surface bacteria, but had no effect on the sequestered bacteria that remained inside the surface cells of the vegetables. Mild bleach reduced the surface coliform bacteria on jarjeer by 84% and reduced the numbers of *E. coli* by 100%. However, upon maceration the disinfected greens released 22X more coliforms and 1,053X more *E. coli* (Table 4). This shows that surface bacteria can be reduced, but the

sequestered bacteria still remain inside the leaves for human consumption. Although there was no testing done for *Salmonella*, it is suspected that the results would be the same. *Salmonella* would survive disinfection and be released once the vegetable was eaten (macerated) just like *E. coli*.

Table 3: Coliform data for the salad greens (Jareer, Parsley, Mint, Spring Onion, and Tabouleh) collected from four stores represented in code as LH, SS, CC, and CF.

Jarjeer greens	**LH**	**SS**	**CC**	**CF**
Presm Coliforms	+	+	+	+
Presm*E. coli*	+	+	+	+
Coliforms	>2,419,600	>2,419,600	>2,419,600	>2,419,600
E.coli	2,600	5,500	5,500	5,500
Salmonella (blk)	++++	+++	+++	+++
Salmonella (mgt)	++++	+++	+++	+++
Parsley	**LH**	**SS**	**CC**	**CF**
Presm Coliforms	+	+	+	+
Presm*E. coli*	+	+	+	+
Coliforms	1,986,300	>2,419,600	>2,419,600	1,299,700
E.coli	None	4,100	44,100	None
Salmonella (blk)	+++	++++	++++	+++
Salmonella (mgt)	+++	++	++++	++
Mint	**LH**	**SS**	**CC**	**CF**
Presm Coliforms	+	+	+	+
Presm*E. coli*	+	+	+	+
Coliforms	1,208,800	>2,419,600	993,150	1,732,900
E.coli	2,000	7,400	137,750	3,150
Salmonella (blk)	+++	+++	++	+++
Salmonella (mgt)	+++	++	+++	++
Spring onion	**LH**	**SS**	**CC**	**CF**
Presm Coliforms	+	+	+	+
Presm*E. coli*	+	+	+	+
Coliforms	>2,419,600	2,419,600	>2,419,600	816,400
E.coli	1,000	14,250	1,000	None
Salmonella (blk)	++	+++	++	++
Salmonella (mgt)	++	+++	++	++
Tabouleh	**LH**	**SS**	**CC**	**CF**
Presm Coliforms	+	++	+	+
Presm*E.coli*	+	++	+	+
CFU	>2,419,600	>2,419,600	>2,419,600	1,046,200
E.coli	2,000	4,850	4,100	1,000
Salmonella (blk)	++	+++	++	++++
Salmonella (mgt)	++	+++	++	++++

Values represent most probable number (MPN) per gram of vegetable. Presumptive tests (Presm) are either + or -- for coliforms and *E. coli*. *Salmonella* plated samples were rated: none = 0 colonies, + = low1-10 colonies, ++ = moderate10-100 colonies, +++ = high >100 colonies, and ++++ = very high >500 colonies. Blk = black *Salmonella sp. 1* colonies; mgt = magenta *Salmonella sp. 2*colonies. Tabouli is a mixture of chopped parsley, mint, spring onion, tomatoes, plus olive oil, and lemon juice.

Table 4: Coliforms and *E. coli* on jarjeer disinfected with mild bleach, rinsed, washed, and then macerated. Sample number (N= 2).

First	Wash Before Bleach	Wash	Water after Bleach	Macerated	
CFU / g	*E. coli* / g	CFU / g	*E.coli* /g	**CFU / g**	*E.coli* /g
235,281	70278,842	0	2,439,200	0	
475,473	10,000	33,649	0	35,895	2,105
Total 710,754	10,702	112,491	…….0	2,475,095	2,105
Av 355,377	5,351	56,246	…….0	1,234,548	1,053
SD ±169,841	±6,575±31,956	0±1,699,393	±1,489		

Growth of Coliforms, *E. coli* and *Salmonella,* in Jarjeer Leaves Stored at Four Temperatures Earlier microscopic examination of sectioned jarjeer leaves showed that bacteria were living in the leaf epidermal cells of fresh produce (Russell *et al.,* 2010). The question to answer here was: are the bacteria simply surviving in the leaf cells or are they multiplying while in transit and storage? The flagellated bacteria observed in fresh leaves were alive and very active, therefore it was reasonable to assume they were surviving and multiplying inside these jarjeer leaf cells. Jarjeer was purchased from local stores and incubated at different temperatures for 20 hours to see if storage was related to increased numbers of coliforms. It needs to be noted that the jarjeer had already been in transit and storage for an unknown period of time before the samples were purchased. The most important result was that the bacteria numbers were increasing in the jarjeer. The results showed that the total coliform numbers increased over a 20 hour period at three of the four temperatures (Figure 1 & Table 5). The most notable and obviously significant increases occurred at 30°, 35°, and 40°C, which is the optimum temperature range for human pathogens and for *Salmonella* (Pui *et al.,* 2011).

Table5: Change in total coliforms (cfu/g) in fresh jarjeer purchased from stores and incubated at four different temperatures for 20 hours. Average weight of sample 5.4g.

Time (hrs)	25°C	30°C	35°C	40°C
0	344,717	33,019	1,046,200	2,441
4	10,673	84,941	207,455	14,904
8	70,094	546,604	379,708	>2,282,642
12	29,727	325,383	>2,419,600	1,333,636
16	12,179	>2,326,539	>2,480,300	>2,326,539
20	30,708	1,805,648	1,773,482	1,752,593
Change	314,009	+1,772,629	+727,282	+1,750,152

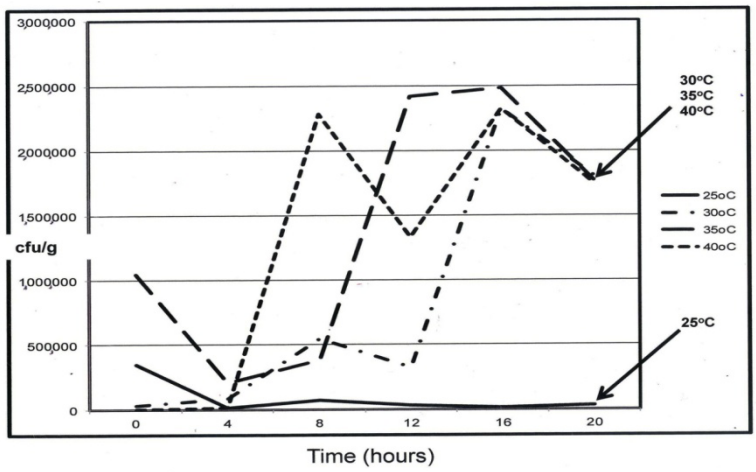

Fig. 1: Changes in amounts of total coliforms cfu/g over time in jarjeer greens stored at four temperatures (25°C, 30°C, 35°C,and 40°C).

Lower growth occurred at 25°C and this cooler temperature appeared to allow slower coliform growth during the 20 hours. *E. coli* numbers declined at each temperature (Figure 2, Table 6), although it may have grown for a while at 35-40°C and then stopped. The rise in *E. coli* between 12 and 20 hours may indicate that *E. coli* was beginning to increase at that temperature once again. Conclusions from this experiment were that total coliforms, which includes *Salmonella*, are multiplying inside the jarjeer leaf cells during storage, especially near the temperature range ideal for human pathogens. *E. coli* may have begun to multiplying at 35-40°C, but its numbers generally declined over time.

Table 6:Change in *E. coli*(cfu/g) in jarjeer purchased from stores and incubated at four different temperatures for 20 hours. Average weight of sample 5.4g.

Time (hrs)	25°C	30°C	35°C	40°C
0	7,547	3,774	0	978
4	962	0	909	3,846
8	948	943	2,061	0
12	909	0	0	0
16	0	1,923	0	0
20	943	2,778	0	0
Change	-6,604	-996	0	--978

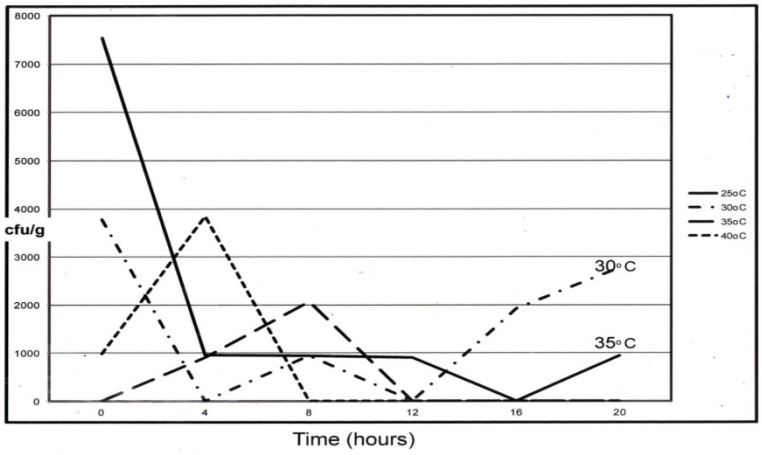

Fig. 2: Changes in amounts of *E. coli* cfu/g over time in jarjeer greens stored at four temperatures (25°C, 30°C, 35°C, and4 0°C).

CONCLUSIONS

1- The total coliform contamination was significantly higher in 2015 (P = 0.026) compared to the results done in 2010. There was no statistical difference between contamination in different samples from the different emirates or stores, all samples were equally contaminated. Organically certified jarjeer was not less contaminated with *E. coli*, when macerated than was non-organic jarjeer.

2- Tests showed that *Salmonella* species were present in all of the types of salad green samples tested (jarjeer, parsley, mint, and spring onions).

3- *Salmonella* and *E. coli* were abundant on the surface of salad greens and were reduced upon disinfection, but disinfection did not reduce the sequestered coliforms and *E. coli* which remained viable inside the leaf epidermis cells.

4- Total coliforms and *E. coli* were high in the main tabouleh salad ingredients

and were also high in the finished product. Tabouleh showed extensive *Salmonella* colony growth and confirmed its abundant presence in 100% of the salads tested.

5- *E. coli* survives disinfection by a commercial product; therefore, it is likely that *Salmonella* is also surviving and could be released once the vegetable was eaten/macerated.

6- Coliforms are multiplying in the jarjeer leaves during storage, especially near the temperature range ideal for *Salmonella* and human pathogen growth.

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REFERENCES

Cetinkaya, F., Cibik, R., Soyutemiz, G. E., Ozakin, C., Kayali, R. and Levent, B. 2008. *Shigella* and *Salmonella* contamination in various foodstuffs in Turkey. Food Control 19:1059-1063.

Clesceri, L. S., Greenberg, A. E. and Eaton, A. d. (Eds). 1999. Standard Methods for the Examination of Water and Wastewater. 20th Ed. American Public Health Association. 1325pp.

Davis, J. G. and Kendall, P. 2007. Preventing *E. coli* from garden to plate. Colorado State University Extension, Nutrition Resources, No. 9.369

Masudi, F. 2014. Food poisoning cases could be higher than reported. Gulf News November 12, p.4.

De Silva, G. D. D., Abayasekara, C.L. and Dissanayke, D R. A. 2013. Freshly eaten leafy vegetables: A source of food borne pathogens? Ceylon J. Sci. 42(2): 95-99.

Montville, T. J. and Matthews, K. R.. 2008. Food Microbiology: An Introduction (2nd Ed.) ASM Press, Washington D.C.

Podolak, R., Enache, E., Stone, W., Black, D. and Elliot, P.H. 2010.Sources and risk factors for contaminaton survival, persistence and heat resistance of *Salmonella* in low- moisture foods. J. Food Protection 10:1780-1955.

Pui, C. F., Wong, W. C, Chai, L. C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M. S., Ubong, A., Farinazleen, M.G., Cheah, Y. K. and Son, R. 2011. *Salmonella:* A food borne pathogen. International Food Res.J. 18: 465-473.

Rao, P. V. 1998. Statistical Research Methods in the Life Sciences, Duxbury Press, Pacific Grove, i-xiv; 889pp.

Russell, D. J., Majid, S. A. and Tobias, D. 2010. The presence of persistent coliform and *E. coli* contamination sequestered within the leaves of the popular fresh salad vegetable "Jarjeer / Rocket" (*Erucasativa* L.). Egypt. Acad. J. biolog. Sci. (G-Microbiology) 2(2): 1-8.

Silva, S. R. P., Verdin, S. E. F., Pereira, D. C., Schatkoski, A. M., Rott, M. B. and Corcao, G. 2007. Microbiological quantity of minimally processed vegetables sold in Portoalegre, Brazil. Brazilian J. Microbiology 38: 594-598.

Venes, D. (Ed.). 2013. Tabor's Cyclopedic Medical Dictionary (22ed), F. A. Davis Co., Philadelphia, 2880pp.