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Escherichia coli **produtora de toxinas Shiga (STEC) e** ***Salmonella*** **spp. em alface**

Shiga toxin-producing ***Escherichia coli*** **(STEC) and** ***Salmonella*** **spp. in lettuce**

Escherichia coli **productora de toxina Shiga (STEC) y** ***Salmonella*** **spp. en lechuga**

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Resumo

As doenças transmitidas por alimentos são relevantes para a saúde pública, especialmente em alimentos contaminados que são consumidos sem cozimento prévio, como alface. O objetivo do presente estudo foi avaliar a contaminação por *Escherichia coli* produtora de toxina Shiga (STEC), *E. coli* e *Salmonella* spp. em amostras de alface. As variáveis foram: tipo de cultura, tipo de estabelecimento e contagem de coliformes. O DNA dos isolados de *E. coli* foi analisado por PCR para a pesquisa de genes de virulência. Isolados confirmados com *Salmonella* nos testes bioquímicos foram submetidos à sorologia com soros anti-*Salmonella*. Trinta amostras de alface foram avaliadas, onze amostras foram positivas para *E. coli* (36,67%) e uma amostra (3,33%) de cultura hidropônica apresentou resultado positivo STEC. Os dois isolados STEC foram positivos para o gene *stx2*. Alfaces cultivadas convencionalmente têm 2,4 vezes maior probabilidade de serem contaminados por *E. coli*. A presença de *Salmonella* spp. foi confirmada em 16,67% (5/30) das amostras. A presença de microrganismos potencialmente patogênicos nas amostras analisadas indica a necessidade de cuidados especiais na preparação de vegetais frescos antes de serem consumidos *in natura*, evitando manuseio excessivo e lavagem com desinfetantes.

Palavras-chave: Contaminação de alface; Segurança alimentar; Verduras; PCR.

Abstract

Foodborne illnesses are relevant to public health, especially in contaminated foods that are eaten without prior cooking, such as lettuce. The objective was to evaluate the contamination by Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* and *Salmonella* spp. in lettuce in commercial food-establishments in the city of Jataí, Goiás, Brazil. The variables were: type of crop, type of establishment and coliform count. The DNA of *E. coli* isolates were analyzed by PCR to the research of virulence genes. Isolates compatible with *Salmonella* in the biochemical tests were submitted for serology with *Salmonella* antisera. Thirty samples of lettuce were evaluated, eleven samples were positive for *E. coli*, (36.67%),

and one sample (3.33%) tested positive for STEC hydroponic crop. The two STEC isolates were positive for the *stx2* gene. Conventionally grown products were 2.4 times more likely to be contaminated with *E. coli*. The presence of *Salmonella* spp. was confirmed in 16.67% (5/30) of the samples. The presence of potentially pathogenic microorganisms in the analyzed samples indicates the need for special care to be taken in preparing fresh vegetables before they are consumed *in natura*, like avoid excessive handling, and washing with sanitizers.

Keywords: Lettuce contamination; Food safety; Green leaves; PCR.

Resumen

Las enfermedades transmitidas por los alimentos son relevantes para la salud pública, especialmente en los alimentos contaminados que se comen sin cocción previa, como la lechuga. El objetivo del presente estudio fue evaluar la contaminación por *Escherichia coli* productora de toxina Shiga (STEC), *E. coli* y *Salmonella* spp. sobre lechuga en establecimientos comerciales de alimentos en la ciudad de Jataí, Goiás, Brasil. Las variables fueron: tipo de cultivo, tipo de establecimiento y recuento de coliformes. El DNA de los aislados de *E. coli* se analizó por PCR para buscar genes de virulencia. Los aislamientos confirmados con *Salmonella* en pruebas bioquímicas se sometieron a serología con sueros anti-*Salmonella*. Se evaluaron treinta muestras de lechuga, once muestras fueron positivas para *E. coli* (36,67%) y una muestra (3,33%) de cultivo hidropónico mostró un resultado STEC positivo. Los dos aislamientos de STEC fueron positivos para el gen *stx2*. Las lechugas cultivadas convencionalmente tienen 2.4 veces más probabilidades de estar contaminadas con *E. coli*. La presencia de *Salmonella* spp. se confirmó en el 16.67% (5/30) de las muestras. La presencia de microorganismos potencialmente patógenos en las muestras analizadas indica la necesidad de un cuidado especial en la preparación de vegetales frescos antes de ser consumidos en la naturaleza, evitando el manejo excesivo y el lavado con desinfectantes.

Palabras clave: Contaminación de lechuga; Inocuidad de los alimentos; Vegetales; PCR.

1. Introduction

Vegetables are often consumed fresh and are distinguished by favor factors such as their high nutritional value as they contain vitamins, minerals and fiber, and also by their adaptability for use in meals. They are highly sought after by consumers who aim to have a healthy diet and achieve fitness and health by eating well (Kłapeć et al., 2016).

Among vegetables, lettuce (*Lactuca sativa*), and all its varieties, are considered one of the most consumed green vegetable and is most often used in salad. Due to their large folded surface area, as in other leafy vegetables, they have a greater capacity for maintaining microorganisms across the different stages of their production up to their arrival on the table of the consumer (Ryu et al., 2014). This presents a high risk of contamination, which may adversely affect the health of consumers. In addition, the use of natural fertilizers such as animal manures and residual irrigation water may facilitate the proliferation and contamination of pathogenic microorganisms in these foods (Decol et al., 2017).

Due the possibility of green leaves contamination, salads may host several species of microorganisms that influence the food's microbiological quality, such as mesophilic bacteria, thermotolerant coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. (Allen et al., 2013). In Brazil, lettuce production accounts for 11% of all vegetable production and around 525,602 tons of letter are produced annually, with a significant increase in production being observed between 2000 and 2011 (Suinaga et al., 2013). With the increase in consumption and the low microbiological quality of this food, the possibility of FBD outbreaks linked to the consumption of lettuce is considered a risk to public health.

In Brazil, the surveillance and microbiological control of fresh vegetable products is limited. Consequently, there is little information about the presence of pathogens in these products and the potential public health risks they pose. The objective of this study was to analyze the contamination by *E. coli*, STEC and *Salmonella* spp. in lettuce, as well as the prevalence of these bacteria in commercial food-selling establishments in Jataí, Goiás, Brazil.

2. Material and Methods

Experimental design

Thirty samples of commercially-sold lettuce purchased in the city of Jataí, Goiás, Brazil were analyzed. The commercial sale of lettuce was the selection criterion for the inclusion of food-selling establishments in this study. The points of sale were visited at random, and the samples were acquired simulating conventional acquisition. They were evaluated by the type of vegetable cultivation used in their production (hydroponic or conventional), the type of establishment from which they were purchased (supermarkets, free fairs, and vegetable gardens) and MPN/g (appropriate or inappropriate), for the evaluation the presence of *E. coli* and *Salmonella* spp. in lettuce samples. The samples were processed according to protocol recommended by brazilian legislation (Brasil, 2003).

Escherichia coli isolation

Isolation of *E. coli* was performed as described by Brasil (2003). A total of 25 g of each sample was added to a plastic stomacher bag together with 225 mL of lactosate broth homogenized in stomacher and thereafter incubated for 24 h, 37 °C, 120 rpm. Following incubation, from this solution a 10 µl loop sown the sample was cultivated using a methylene blue agar and incubated again for 24 h at 37 ºC. After 24 h, of each plate up to five colonies were *E. coli* suggestive colonies were selected for confirmation by the biochemical tests TSI, Indol, Methyl Red, Voges Proskauer and Citrate (IMVic). *E. coli* was stored on agar conservation and lyophilized for the subsequent realization of genotypic characterization tests.

Salmonella spp. isolation

Isolation of *Salmonella* spp. was performed as described by Brasil (2003). A total of 25 g of the sample was suspended in 225 mL of buffered saline solution (pH 7.6) with the objective of promoting pre-enrichment. From the enrichment medium, 1 mL was inoculated in tubes containing 10 mL of Rappaport Broth and tubes with 10 mL of Selenite Sistine Broth incubated at 41 °C for 24 h to 30 h in a water bath. From the selective broth, the samples were then plated on *Salmonella-Shigella* agar (SS) and bright green agar phenol lactose sucrose (BPLS), where three to ten colonies were selected and submitted for biochemical identification tests (TSI, Indole, Citrate, MR-VP, urease). Isolates compatible with *Salmonella* spp. in the biochemical tests were submitted for serology with *Salmonella* antisera (antigens O groups A, B, C, D, E and Vi. Flagellar antigens a; b; w; d; i; 1, 2, 5.).

Most Probable Number (MPN)

Presumptive tests were performed for total coliforms and thermotolerant coliforms according to Brasil (2003). The results were expressed as MPN/g (appropriate or inappropriate) for consumption.

PCR detection of _Escherichia coli_ virulence genes

DNA extraction of *E. coli* isolates was performed by thermal lysis. Following extraction, the DNA were analyzed by PCR to identify the possible STEC, using a solution of a final volume of 25 µL, consisting of 2.5 µL of DNA, 10 pmoL of each primer, 10 mM dNTP, 50 mM $MgCl_2$, buffer 10× and 1 U of Taq DNA polymerase (Invitrogen, USA).

Amplification conditions were performed with an initial denaturation at 94 °C for two minutes, followed by 35 cycles of 94 °C for one minute (denaturation), 55 °C for one minute (annealing primers) and 72 °C for one minute (extension). The final extension was performed for ten minutes at 72 °C. The protocol for the amplification conditions was previously described by Paton and Paton (1998) as *stx2* and *rfbO157*; by Gannon et al. (1997) as *fliCh7*; by Wang et al. (2002) as *stx1* and *eae*; by Paton and Paton (1998) as *saa*; by Blanco et al. (2004) as *ehxA*; and by Yamamoto et al. (1995) as *cnf1* (Table 1). *E. coli* O157:H7 (ATCC EDL 933) was used as a positive control and *Klebsiella pneumoniae* (ATCC BAA 1705) was used as a negative control for PCR. The PCR product was loaded onto a 2% agarose gel containing ethidium bromide (0,5 μg/μL) in a 1 × TAE running buffer (89 mM Tris, 89 mM ácido bórico; 2,5 mM de EDTA, pH 8,0) and electrophoresed at 70 V for 90 min. For the strains positive for *stx1* and/or *stx2*, the virulence factors *eae*, *ehxA*, *saa* and *cnf1* were also evaluated.

Table 1. PCR primers used for detection of virulence factors genes of STEC.

Gene	Sequence (5' to 3')	Product size (bp)	Reference
eae	ATGCTTAGTGCTGGTTTAGG	248	Wang et al. (2002)
	GCCTTCATCATTTCGCTTTC		
rfbO157	CGGACATCCATGTGATATGG	259	Paton and Paton (1998)
	TTGCCTATGTACAGCTAATCC		
fliCh7	GCGCTGTCGAGTTCTATCGAGC	625	Gannon et al. (1997)
	CAACGGTGACTTTATCGCCATTCC		
saa	CGTGATGAACAGGCTATTGC	119	Paton and Paton (1998)
	ATGGACATGCCTGTGGCAAC		
ehxA	GGTGCAGCAGAAAAAGTTGTAG	1551	Blanco et al. (2004)
	TCTCGCCTGATAGTGTTTGGTA		
cnf1	AAGATGGAGTTTCCTATGCAGGAG	498	Yamamoto et al. (1995)
	CATTCAGAGTCCTGCCCTCATTATT		
stx1	TCTCAGTGGGCGTTCTTATG	338	Wang et al. (2002)
	TACCCCCTCAACTGCTAATA		
stx2	GGCACTGTCTGAAACTGCTCC	255	Paton and Paton (1998)
	TCGCCAGTTATCTGACATTCTG		

Statistical analysis

The data were computed using the Statistical Analysis System - SAS v.9.3 (2010) program to 5% probability. The Fisher´s Exact Test was used separately for each microorganism and variable. The microorganisms evaluated were *E. coli* and *Salmonella* spp. The variables were: Type of crop (1. Hydroponic or 2, Convetional), type of establishment (1. Supermarkets or 2. Vegetable gardens/free fairs) and coliform count (1. Inappropriate for consumption (i.e. more than 10^2 MPN/g at 45 °C) or 2. Appropriate for consumption (i.e. maximum 10^2 MPN/g at 45 ºC).

3. Results

Analysis revealed that 43.3% (13/30) of the samples examined were of the conventional type and 56.7% (17/30) were of the hydroponic type, and 60% (18/30) were purchased from supermarkets and 40% (12/30) from free fairs/vegetable gardens. In terms of the thermotolerant coliform count (MPN), 86.7% (26/30) of the samples were considered inappropriate for consumption according to the National Sanitary Surveillance Agency parameters presented above, and just 13.3% (4/30) were found to be within permissible parameters, values ranged from absent to 1.6×10^5 MNP (Table 2).

Table 2. Analyzed lettuce samples (*Lactuca sativa*).

Samples	Type of crop	Type of establishment	TC	TTC	*Salmonella* spp.	*Escherichia coli*
			MPN/g			
1	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
2	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
3	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
4	Hydroponic	Supermarket	$1,6\times10^5$	1.6×10^5	Absent	**Present**
5	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
6	Conventional	Vegetable gardens /fairs*	1.6×10^5	1.6×10^5	Absent	**Present**
7	Hydroponic	Vegetable gardens /fairs*	7×10^2	5.4×10^3	Absent	Absent
8	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
9	Hydroponic	Vegetable gardens /fairs*	1.6×10^5	1.6×10^5	Absent	Absent
10	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
11	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	**Present**
12	Conventional	Supermarket	1.6×10^5	1.6×10^5	**Present**	**Present**
13	Conventional	Supermarket	9×10^3	1.6×10^5	**Present**	Absent

14	Hydroponic	Supermarket	3.6×10^3	$9 \times 10^{2**}$	Absent	Absent
15	Hydroponic	Supermarket	Ausente	Ausente**	Absent	Absent
16	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	**Present**	Absent
17	Conventional	Vegetable gardens /fairs	8×10^2	9×10^3	Absent	Absent
18	Conventional	Vegetable gardens /fairs	8×10^2	$2.9 \times 10^{2**}$	Absent	**Present**
19	Conventional	Vegetable gardens /fairs	1.6×10^5	$2.9 \times 10^{2**}$	Absent	**Present**
20	Conventional	Supermarket	1.6×10^5	$4.5 \times 10^{2**}$	**Present**	**Present**
21	Conventional	Vegetable gardens /fairs *	1.7×10^2	$1 \times 10^{2**}$	Absent	Absent
22	Hydroponic	Supermarket	1.6×10^5	9×10^3	Absent	**Present**
23*	Hydroponic	Vegetable gardens /fairs*	1.6×10^5	$1.3 \times 10^{2**}$	Absent	**Present**
24	Hydroponic	Supermarket	1.6×10^5	Absent **	Absent	Absent
25	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
26	Hydroponic	Supermarket	1.6×10^5	1.6×10^5	Absent	Absent
27	Conventional	Vegetable gardens /fairs*	1.6×10^5	8.2×10^4	**Present**	**Present**
28	Conventional	Vegetable gardens /fairs	1.6×10^5	1.6×10^5	Absent	Absent
29	Conventional	Vegetable gardens /fairs	1.6×10^5	1.6×10^5	Absent	Absent
30	Conventional	Vegetable gardens /fairs	1.6×10^5	1.6×10^5	Absent	**Present**

Analyzed lettuce samples (*Lactuca sativa*). TC: total coluiforms; TTC: t thermotolerant coliforms (*) Samples bought at a fair. (**) Positive sample for STEC; Permissible counts of thermotolerant coliforms (TTC) (BRASIL, 2001).

The presence of *E. coli* was confirmed in 11 samples, representing a frequency of 36.7% (11/30) and there was no difference for the type of cultivation (P=0.2556), and for the type of establishment P=0.6272) e MPN (P=0.7046). Of these contaminated samples according the type of cultivation 36.36% (4/11) were from hydroponic lettuces e 63.64% (7/11) were from conventional cultivation, corresponding to 23.5% (4/17) were from hydroponic lettuces and 53.9% (7/13) were from conventional cultivation. Evaluating the effect of cultivating lettuce isolates in the final model, conventionally grown products were 2.4 times more likely to be contaminated with *E. coli* (Table 3),

According to the commercial establishment in which the samples were purchased; we observed that 27.8% (5/18) of the supermarket samples and 50% (6/12) of the free fairs/vegetable gardens samples were contaminated (MPN/g), 38.5% (10/26) inappropriate and 25% (1/4) appropriate. The frequency of STEC in the total analyzed lettuce was 3.33% (1/30). This single positive sample was acquired at a free fair. Among the isolates *E. coli*, 7.14% (2/28) were confirmed as STEC (*stx1*$^{+}$ or *stx2*$^{+}$). The two *E. coli* STEC were positive for the *stx2* gene, but the genes *ehxA*, *flicH7*, *rfbO15*, *eae* and *saa* were not found in these strains.

Table 3. Odds ratio of the estimates of the final multivariate dichotomous logistic regression model for the presence of Escherichia coli in the hydroponic crop lettuce samples.

Variable		**Odds ratio**	**IC**	**P-value**
Type of crop	Conventional x hydroponic	2.420	1.116 a 5.247	0.0252

The presence of *Salmonella* spp. was confirmed in 16.7% (5/30) of the samples. For the presence of *Salmonella* spp., there was also no difference for the type of crop (P=0.0131), and for type of establishment (P=0.0283), MPN (P=0.1545) (Table 5). A total of 30.8% (4/13) of the conventionally cultivated lettuce were positive, while, of the hydroponics, 5.9% (1/17) were contaminated. Among the samples that were contaminated, 22.2% (4/18) were acquired from supermarkets and 8.3% (1/12) from free fairs/vegetable gardens (Table 5). Of the 22 samples found to be unsuitable for consumption because they contained higher than permitted values of thermotolerant coliforms (TtC), 15.4% (4/26) were contaminated with *E. coli*, and 25% (1/4) with *Salmonella* spp. However, of the eight samples with permissible values, 50% were contaminated with *E. coli*, and 12.50% with *Salmonella* spp.

Table 4. Presence of *Salmonella* spp. in lettuce samples according to the type of crop, MPN and the type of food-selling establishment the lettuce was purchased from.

***Salmonella* sp.**	**Type of crop**	**MPN**	**Type of establishment**
P-Value	0.0131	0.1545	0.0283

Table 5. Odds ratio of the multivariate dichotomous logistic regression for the presence of *Salmonella* spp. in hydroponic crop lettuces samples and samples purchased from supermarkets.

Variable			***Odds ratio***	**IC (95%)**	**P-value[1]**
Type of crop	Conventional Hydroponic	×	39.883	1.945 a 817.726	0.0168
Type of establishment	Vegetable gardens/fairs Supermarkets	×	0.041	0.002 a 0.860	0.0397

[1] Wald test.

The presence of 55 *E. coli* strains was confirmed. Of these, 36.4% (20/55) were present in the hydroponic lettuce and 63.6% (35/55), in the conventionally cultivated lettuce. When we analyzed the isolates obtained according to their purchase location, 45.5% (25/55) and 54.5% (30/55) came from supermarkets and free fairs/vegetable gardens, respectively.

4. Discussion

Brazilian legislation does not provide for a maximum limit for the presence of *E. coli* in vegetables; however, it establishes the maximum limit of 10^2 MPN/g for coliforms (Brasil, 2003). *E.* coli is included in the coliform group and 63.64% (7/11) of samples that tested positive for *E. coli* in this study were found to be beyond safe limits for coliforms, that is, testing returned results above the limit of 10^2 MPN/g. Mora et al. (2011) evaluated 200 vegetable samples and isolated *E. coli* in 195, yet all showed satisfactory quality, that is, that were within permissible coliform limits. According to Gomes Neto et al. (2012), the presence of several strains of *E. coli* suggests that this contamination may be of fecal origin, and also indicates a greater probability of contamination with other pathogenic bacteria; therefore, these vegetables are kept in unsanitary conditions, representing a risk to consumers' health.

In the present study, 36.7% (11/30) of the lettuce samples were contaminated with *E. coli*. This result is similar to that reported Khatib et al. (2015), who found *E. coli* contamination in 36% of the lettuces analyzed in Lebanon, but lower than the figures reported by Greve et al. (2015), which found *E. coli* in 98.2% of 720 lettuce samples purchased in the Upper Midwest of the United States The samples were sold commercially in Campinas, São Paulo. In Rio de Janeiro, Brandão et al. (2014) found *E. coli* in 70% of fresh lettuce samples, a far high result than the one reported in our study.

Stx family is divided into two groups: *stx1* and *stx2*. Stx variants include three stx1 subtypes and seven *stx2* subtypes. However, *stx2* is considered clinically to be the most important type and is more likely to cause Uremic Hemolytic Syndrome in humans. In addition, it is reported that the development of infection by strains that contain *stx2* is more likely than by strains containing *stx1* or both *stx1* and *stx2* genes. The STEC from the lettuce samples were positive only for the *stx2* gene, which shows that lettuces sold in Jataí, Goiás, Brazil, can carry microorganisms that are potential pathogenic to humans. It was confirmed that this isolate belonged to the STEC group and not EHEC, as it detected the *stx2* gene, but did not present other virulence factors investigated and related to the EHEC group (Wang et al., 2002).

In a study in Iran with 100 lettuce samples, eight STEC were found, with the presence of *stx1* genes identified in 37.5% (3/8) and *stx2* in 62.5% (5/8). All samples found to be positive for *stx2* also tested positive for *rfbO157* and *flicH7* genes Mazaheri et al. (2014). Moreover, Khatib et al. (2015) found STEC in 17.9% (7/39) of the isolates examined, with two of the positive samples being from lettuces. These studies demonstrate a higher percentage of STEC than the present study, which reports a prevalence of 3.33% (1/30).

The presence of positive strains for the *stx1*, *stx2*, and *eae* genes in vegetables is highly variable (Dutta et al., 2014). This variability of results may be related to the methodologies used, to the difficulty in standardization of methodologies and the different *E. coli* populations circulating between animals and in food contaminated by feces, mainly bovine manure.

In the current study, the presence of STEC in lettuce produced using hydroponic systems may be related to the use of contaminated water. Studies confirm the contamination of water with STEC and other pathogenic *E. coli*, indicating water as a potential vehicle for these agents and demonstrating the survival and multiplication of these agents in study samples (Ceuppens et al., 2015). According to Wang et al. (2002), *E. coli* O157: H7 has great survival capacity in water and can remain in pond water for up to 13 weeks at 15 ºC. The

positive result for STEC in the present study agrees with previous research that has detected this pathogen in cattle and water from rural properties in the state of Goiás, revealing that these animals are important reservoirs of this microorganism and that they can excrete it in their feces, contaminating water, plantations and, consequently, vegetables and fruits (Ferreira et al., 2014).

The presence of *Salmonella* spp. in 16.67% (5/30) of the analyzed samples shows the low microbiological quality of lettuces marketed in the city of Jataí. A study examining vegetables from Colombia, the Dominican Republic, Guatemala, Mexico and the United States found no vegetables, including lettuce, contaminated with *Salmonella* spp. (Allen et al., 2013). In Brazil, Brandão et al. (2014) not found *Salmonella* spp. in any analyzed lettuce sample. Other studies carried out in different regions of the world have reported a lower prevalence of the microorganism than the present study or have reported no presence of the microorganism at all (Ceuppens et al., 2015; Kłapeć et al., 2016).

Allen et al. (2013) show that between 1995 and 2011 there were 54 FBD outbreaks worldwide specifically linked to the consumption of lettuce and other leafy vegetables, causing illness in more than 2,426 patients in Canada, Denmark, the United Kingdom and the United States, with *Salmonella* spp. among the etiological agents associated with these outbreaks. According to Gomes-Neto et al. (2012) between the year 2000 and 2012, *Salmonella* spp. was the most important pathogen associated with FBD outbreaks in Brazil.

The type of crop revealed differences in the presence of *E. coli* and *Salmonella* spp. in the samples, confirming that the type of crop may influence the level of contamination. In this work, conventionally cultivated crops were more likely to be contaminated (Gomes Neto et al., 2012). This can be explained by Marine et al. (2015) who argue that conventional cultivation may favor the growth of microorganisms and even cross-contamination between the water and organic fertilizers used.

According Ceuppens et al. (2015) the quality of irrigation water used in this type of crop can be both a source and a route of microbial contamination. In addition, the type of irrigation used may influence microbial growth. In the same study, the use of flood irrigation increased the prevalence of *Salmonella* spp. in fresh vegetables. In addition, the use of manure-type fertilizer as organic fertilizer and the combination of livestock and production of fresh vegetables are also considered potential risk factors.

It is important to emphasize that soil is an important source of contamination, and, in contrast to a hydroponic cultivation system, in a conventional cultivation system plants have direct contact with the soil, which may explain the different data found in this work (Gomes

Neto et al., 2012). Furthermore, the prevalence of these pathogens in the agricultural environment may vary according to the climatic characteristics of a region, such as increased temperatures and flooding, which may have a positive or negative impact on the survival of coliforms and, consequently, may increase or decrease the risk of contamination of fresh vegetables and associated FBD outbreak frequency.

The differences observed between the two types of crop was similar to the differences reported by Gomes Neto et al. (2012) who concluded that lettuces from conventional and organic crop systems presented poor hygienic-sanitary quality and higher contamination than hydroponic lettuces. Moreover, their results showed that the cleaning of lettuces with sterile water was sufficient to reduce the number of microorganisms to safe levels in hydroponic samples; therefore, greater care regarding hygiene is required during all stages of plant production, such as the adoption of good agricultural practices.

In conclusion, the presence of pathogenic microorganisms in the analyzed samples indicates the need for special care to be taken in the sanitization of fresh vegetables before they are consumed *in natura*. Although the present study did not isolate O157: H7 strains in lettuce, other pathogenic microorganisms, such as *Salmonella* spp., *E. coli*, STEC, coliforms, thermotolerant coliforms and mesophilic bacteria were found.

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