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A microbiological survey on tomatoes (*Lycopersicon esculentum* Mill) marketed in Rio de Janeiro, Brazil

Avaliação microbiológica de tomates (*Lycopersicon esculentum* Mill) comercializados no Rio de Janeiro, Brasil

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ABSTRACT

Fresh produce can be a possible source of microbiological contamination. In the past 20 years, several salmonellosis outbreaks due to the consumption of tomato have been reported, mainly in the USA. Organic raw vegetables pose a risk for the transmission of foodborne pathogens since they are often cultivated using manure as a fertilizer. The aim of this study was to conduct a survey of the presence of *Salmonella* spp, total coliforms and *Escherichia coli* on the surface of tomatoes from two different production systems. A total of 262 samples of organic and conventional tomatoes were collected from supermarkets and open street markets in the city of Rio de Janeiro, Brazil and analyzed for these microorganisms. To increase the probability of detecting *Salmonella* spp, we used two different detection methods: the traditional method from the Bacteriological Analytical Manual-Food and Drug Administration (BAM-FDA) and the Mini-Vidas-SLM-bioMérieux. Total coliforms were detected in the range of 1 to 4 log10 CFU/g, *E. coli* was found in only three samples (2 conventional and 1 organic) and *Salmonella* was absent in all of the analyzed samples. The results demonstrate that all of the samples were in agreement with the Brazilian legislation for *Salmonella* spp.

KEYWORDS: *Salmonella* spp.; Tomatoes; Organic; Conventional; *Escherichia coli*; Sanitary Surveillance

RESUMO

Produtos agrícolas frescos podem ser uma fonte possível de contaminação microbiana. Nos últimos 20 anos diversos surtos de salmonelose devido ao consumo de tomate têm sido relatados principalmente nos EUA. Vegetais orgânicos frescos oferecem risco na transmissão de patógenos de origem alimentar devido a serem frequentemente cultivados utilizando esterco como fertilizante. O objetivo deste estudo foi realizar uma pesquisa para verificar a presença de *Salmonella* spp, coliformes totais e *Escherichia coli* na superfície de tomates provenientes de dois sistemas diferentes de produção. Um total de 262 amostras provenientes da produção orgânica e convencional de tomates foram obtidos de supermercados e feiras na cidade do Rio de Janeiro, Brasil e analisados para estes microrganismos. Para aumentar a probabilidade de detecção de *Salmonella* spp., dois diferentes métodos de análise foram utilizados, um tradicional do Manual de Análises Bacteriológicas da Administração de Alimentos e Drogas dos Estados Unidos da América e o Mini Vidas-SLM-bioMérieux. Coliformes totais foram detectados na faixa de 1 a 4 log10 UFC/g, *E. coli* foi encontrada em somente três amostras (duas convencionais e uma orgânica) e *Salmonella* estava ausente em todas as amostras analisadas. Os resultados mostraram que todas as amostras estavam de acordo com a legislação brasileira para *Salmonella* spp.

PALAVRAS-CHAVE: *Salmonella* spp.; Tomates; Orgânico; Convencional; *Escherichia coli*; Vigilância Sanitária

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INTRODUCTION

It is widely accepted that a healthy diet is an important factor in preventing chronic disease, improving energy balance and managing weight, so the consumption of fruits and vegetables has become a global health priority[1,2].

Tomatoes represent a reservoir of potentially healthy micronutrients such as ascorbic acid, vitamin E, minerals (potassium) and antioxidants[3,4], and the dietary intake of this fruit has shown to be associated with a decreased risk of chronic diseases such as cancer and cardiovascular disease[1,5]. Despite the health benefits, contamination of fresh produce is of special concern because such produce is likely to be consumed raw, without any type of microbiologically lethal processing, thus posing a potential health risk[6]. Raw tomatoes have been recognized as potential vehicles for human salmonellosis since *Salmonella* Javiana was first identified as an etiological agent of a multi-state outbreak in 1990[7] and several foodborne illness outbreaks related to tomatoes have been published lately[8,9,10].

Surveys of raw fruits and vegetables demonstrate that there is a potential for a wide range of these products to become contaminated with microorganisms, including human pathogens[11]. To avoid the adverse human health and economic consequences of foodborne illness, all food must be produced following practices that result in products that are safe for consumption – this is true for organic and conventional cultivation[12,13]. The worldwide demand for organic food products continues to expand rapidly, especially in developed countries. This demand is fueled in part by increased consumer awareness of the link between health and diet. However, some questions have been raised about the possibility of an increased risk of microbiological contamination in foods produced in the organic system, mainly due to the type of fertilizer employed[14,15].

Conventional agriculture uses herbicides, pesticides, and chemical fertilizers that have the potential to pollute the surrounding land, air and water. Organic agriculture tries to avoid using these herbicides, pesticides, and chemical fertilizers and promotes an environmentally friendly approach to agriculture. Instead of relying on herbicides, pesticides and chemical fertilizers, organic agriculture promotes a whole system approach to managing weeds, pests and nutrients[16]. In both systems, manure is commonly applied as a fertilizer to fields used for vegetable production; therefore, the application of untreated manure, which may contain pathogenic bacteria such as *Salmonella* spp, can contaminate the surrounding soil, irrigation water and plants, presenting the risk of contamination of the growing vegetables[17].

Despite the various studies in the literature assessing the microbiological quality of vegetables produced in Brazil[18,19,20,21,] and other countries[22,23,24], the number of studies comparing the microbiological quality only for tomatoes is almost inexistent.

An important issue to consider when conducting food safety studies is the bacteria antibiotic resistance. Zurluh et al.[25], Said et al.[26], Kim et al.[27] and Hoek et al.[28] showed that the extended-spectrum *Β* lactases (ESBLs) bacteria represent a growing problem involving food safety and environmental integrity. The authors reported that fresh produce could be contaminated with these bacteria, suggesting that mandatory guidelines should be established in order to ensure consumer and public health worldwide.

Also studies related to the prevalence of enterococcus in fresh produce are important to be conducted, since they can be implicated in severe multi resistant nosocomial infections and are widely distributed in nature, as showed by a study conducted by Gomes et al.[29].

Although those studies are very important, the main purpose of the present study was to be in accordance with the Brazilian legislation concerning the presence or absence of *Salmonella* spp. and *Escherichia coli* on the surface of conventional and organic tomatoes from markets in the city of Rio de Janeiro, Brazil.

METHODS

Sampling

From February 2011 to October 2012 a total of 262 tomatoes (149 from conventional and 113 from organic production) were collected from street markets and supermarkets located in the city of Rio de Janeiro, RJ, Brazil.

Tomatoes were collected in units of five or more and each fruit was individually analyzed. Samples were from 27 different locations: 12 from organic production and 15 from conventional production.

All samples were taken to the laboratory in sterile plastic bags and kept under refrigeration until tested.

Sample preparation

With the assistance of sterilized forceps, knives and dishes, tomatoes were individually peeled and peels were placed inside stomacher bags, weighed and added to sterile 0.1% Buffered Peptone Water (BPW – Difco, France) in a ratio of 1:9. The samples were then homogenized in a stomacher for 5 minutes at 200 rpm.[30] Three additional decimal dilutions were carried out using the same diluent.

Microbiological analysis

Total coliforms and *E. coli* were enumerated using the Petrifilm TM method[31] and the results were expressed as colony-forming units per gram (CFU/g). The occurrence of *Salmonella* spp was evaluated simultaneously by a traditional method of Food and Drug Administration- Bacteriological Analytical Manual[32] and by a rapid method of mini Vitek Immuno Assay Diagnostic System (Mini-Vidas-SLM)- bioMérieux and the results were expressed as presence or absence of *Salmonella* spp.

Statistical analysis

Results expressed as CFU/g were converted to decimal logs and subjected to the Mann-Whitney test to determine whether the levels of contamination of conventional and organic tomatoes and the levels of contamination of tomatoes collected from supermarkets and open markets differed significantly ($p < 0.05$). The software R (R Core Team, 2013) was used for statistical analysis.

RESULTS AND DISCUSSION

To evaluate the surface microbiological quality of tomatoes from conventional and organic production collected from street markets and supermarkets, analyses for *Salmonella* spp, total coliforms and *E. coli* were performed.

Salmonella spp was not isolated from any of the 262 analyzed samples, which is in agreement with the Brazilian legislation[33]. Similar results were reported by Bohaychuk et al.[34], Mukherjee et al.[22] and Gorny[35].

Meanwhile, Wells and Butterfield[36] collected 48 different fruits and vegetables in New Jersey, USA, between 1992 and 1995, and *Salmonella* spp was confirmed in 42% of the tomatoes analyzed. Arthur et al.[23] surveyed 141 fresh market tomatoes from Ontario, Canada and only one sample was positive for *Salmonella*. In another study conducted by Badosa et al.[6] a group of 72 fruits from retailers in Girona, Spain, including tomatoes and green and red peppers, were analyzed and *Salmonella* was found in one sample of tomato.

Table 1 shows the counting results for total coliforms in both conventional and organic cultivations of Brazilian tomatoes. Total coliform counts varied from 1 to 6 log10 CFU/g for organic tomatoes and 1 to 5 log10 CFU/g for conventional tomatoes. Most samples had counts ranging from 1 to 4 log10 CFU/g. This group of microorganisms can be present in vegetables, soil and feces and the presence of this group of bacteria in food is not necessarily a sign of fecal contamination. Concentrations of $3.1 \times 10^3 \pm 3.2$ CFU/g were found on tomatoes analyzed by Ameyapoh et al.[37], which is consistent with the results of this study. Viswanathan and Kaur[38] reported coliform counts ranging from 6.0×10^3 to 1.0×10^7 CFU/g, which are higher than the levels found in this study. Meanwhile Seow et al.[39] studied the enumeration of coliforms in tomatoes and the results were in the range of 0.3 to 3.0 log CFU/g for thirteen samples, which is also lower than the levels observed in our study.

In this study, *E. coli* was detected in three samples – two conventional and one organic.

Bohaychuk et al.[34] collected tomatoes from farmers' and public markets in Alberta, Canada and examined 80 and 567 tomatoes, from organic and conventionally production, respectively; *E. coli* was absent in all samples. Meanwhile Kokkinakis and Fragkiadakis[40], in the city of Crete, Greece, analyzed 60 tomatoes from six mass catering establishments and all samples were positive for *E. coli*.

Forslund et al.[41] also examined tomato samples from Crete and reported that only two out of 84 tomato surface samples contained *E. coli* (mean: 2700 CFU/g) while 36 tomato surfaces from Italy were free of *E. coli*. In both locations, the *E. coli* incidence was low and as stated by the author, although tomatoes may appear as low risk crops for fecal contamination due to their smooth surface, the reports of human disease outbreaks associated with consumption of tomatoes underline the need for further investigations.

The mean microbial counts of the different organic and conventional tomatoes in this study were compared to verify whether they differed significantly from one another ($p < 0.05$) (Table 2). The organic and conventional tomatoes showed significant differences in the total coliform counts according to the Mann-Whitney test ($p = 0.04997$).

The mean microbial counts of the tomatoes collected from supermarkets and open markets were compared to verify whether they differed significantly ($p < 0.05$) (Table 3). Tomatoes collected from supermarkets and open markets showed

Table 1. Level of total coliforms in organic and conventional tomatoes.

Count interval *	Total coliforms (%)	
	Conventional	Organic
10^1 - 10^2	36.91	33.63
10^2 - 10^3	35.57	23.89
10^3 - 10^4	20.13	17.70
10^4 - 10^5	7.38	17.70
10^5 - 10^6	0	7.08
10^6 - 10^7	0	0
$>10^7$	0	0
Total **	149	113

*UFC/g.
**Analyzed samples.

Table 2. Microbial counts for total coliforms present on tomatoes, based on the 202 cropping system used.

Cropping system	Total coliforms
Conventional	2.42 ± 1.04*
Organic	2.81 ± 1.42*

Results expressed as mean ± SD (log10CFU/g).
Statistical differences were determined by the Mann-Whitney test.
*$p < 0.05$.

Table 3. Microbial counts for total coliforms based on the origin of tomatoes.

Origin	Total
Supermarket	2.85 ± 1.09*
Open market	2.23 ± 1.32*

Results expressed as mean ± SD (log10CFU/g).
Statistical differences were determined by the Mann-Whitney test.
*$p < 0.05$.

significant differences in the total coliform counts according to the Mann- Whitney test ($p < 0.00001$).

CONCLUSION

In conclusion, the results of this study show that organic and conventional tomatoes marketed in the city of Rio de Janeiro, RJ, Brazil, were in accordance with the Brazilian legislation for *Salmonella* spp; only a small percentage (1.1%) of samples analyzed for *E. coli* were present, which may indicate fecal contamination. The difference in coliform levels between the two productions systems may be due to the general hygiene applied for each crop during food production or handling.

More studies related to the incidence of foodborne pathogens and antibiotic resistant bacteria on fresh produce should be conducted in order to change the Brazilian legislation.

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Conflito de Interesse

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