Article

Presence of enterobacteria in agricultural inputs in La Comarca Lagunera

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Abstract

The global food trade situation forces exporting countries to strengthen their control systems and adopt and monitor safety control strategies based on the risk of contamination. The objective of this research was to isolate enterobacteria in various inputs used in agricultural production in La Comarca Lagunera and to determine the risk involved in its use in agricultural activities. Samples were microbiologically analyzed from different sources of water, manures and soil, used in the agricultural production of La Comarca Lagunera, Mexico, during the years 2014-2016. *Klebsiella* spp. to was identified as the main risk factor of bacterial contamination in water that is used for irrigation of different crops with a prevalence of 46.95%. In the soil samples, only *Klebsiella* spp. with a prevalence of 60%, while in samples of bovine manure *E. coli* (30%) and *Klebsiella* spp. (40%), *Salmonella* spp. (14%) in goat manure, and *Salmonella* spp. (100%) in chicken manure. The above represents a risk factor for the health of the final consumer, in the case of producers who intend to export their agricultural products, they would reduce competitiveness and could close international markets. Further investigation is necessary along the fruit and vegetable production chain in order to reduce this contamination.

Keywords: *E. coli*, *Klebsiella* spp., *Salmonella* spp., agricultural safety.

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Introduction

Fruits and vegetables provide essential nutrients such as vitamins, fiber, minerals and have multiple health benefits, however, they are also recognized as potential vehicles of foodborne diseases (ETA) (Mesbah *et al*., 2017). It is currently considered that to ensure the food safety of agricultural products it is necessary to prevent contamination in the field and minimize cross contamination during post-harvest management, however, preventing contamination in fields or greenhouses is a challenge and even Good agricultural practices (GAP) are insufficient to ensure that human pathogens are not introduced into the chain of agricultural products (Francis *et al*., 2012).

The open-field production of horticultural products makes them susceptible to contamination by multiple sources. Several reviews have been made on the source of contamination with soil, water, biological amendments and the activity of wild animals, all considered as routes by which human pathogens can be introduced (Warriner *et al.*, 2009; Olaimat and Holley, 2012; Goodburn and Wallace, 2013; Nuesch-Inderbinen and Stephan, 2016).

Even in greenhouses, which are considered closed environments, pathogens such as *Salmonella* can be established (Holvoet *et al*., 2014). It is documented that enteric pathogens can survive in manure, water and soil for prolonged periods (Franz *et al*., 2005; Ferens and Hovde, 2011; Brandl *et al*., 2013) and bind tenaciously to a variety of seeds of plants, roots and leaves (Tyler and Triplett, 2008; Teplitski *et al*., 2011).

In Mexico, vegetable production represents 1.6% of the total value of national agricultural production, and in La Laguna of Coahuila and Durango, it amounts to 16.12 billion pesos (SIAP, 2016). In recent years, fresh fruits and vegetables have attracted the attention of researchers not only because of the benefits that their consumption brings to the health of people, but also because of their association with various outbreaks of gastrointestinal diseases registered in the last decade (Xuan *et al*., 2000). In recent years, the origin of foodborne diseases (ETA) has changed, new pathogens or more aggressive and antibiotic resistant strains have emerged (Rojas-Herrera and González-Flores, 2006).

SENASICA promotes the implementation of pollution risk reduction systems (SRRC), with the aim of reducing the risk of contamination in the production of fruits and vegetables (SAGARPA-SENASICA, 2018).

Thus, the implementation of good agricultural practices (GAP) in the production units of vegetables should reduce the risks of contamination and allow compliance with the microbiological limits and pesticide residues established in national and international regulations. The SRRC have been implemented in recent years mainly in export crops such as chili, tomato, onion and tomato (Callejas *et al*., 2006). Only in 2016, the cultivated area of these 3 vegetables in the lagoon region was more than 9639.03 ha, generating a production value of $16 535 million pesos (SIAP, 2016).

The Comarca Lagunera is a region with great agricultural and livestock activity and due to the great cattle and poultry production that exists, 1 million tons of bovine manure and chicken manure are produced each year with a great potential to be used as organic fertilizer in agricultural production (Ramírez *et al*., 2016). However, these inputs represent a high risk of pathogen transmission (Peralta-Veran *et al*., 2016).

According to Sandoval and Colli (2004), another of the risk factors of microbial contamination of horticultural products is the practice of irrigation with rounded water from natural tributaries, contaminated wells and the use of untreated wastewater. In the Lagunera Region 60% of the horticultural production is irrigated with water from the water waterwheel and 40% with river water (SIAP, 2016). Although the use of wastewater for irrigation provides a large number of nutrients, some studies indicate that the use of untreated water for agricultural irrigation is the practice that most influences the reduction of the sanitary quality of fruits and vegetables (Cazárez *et al*., 2004; Allende *et al*., 2008).

The ETA is a major problem worldwide because they cause high morbidity and mortality, according to the world health organization, it is estimated that each year around 600 million people in the world are ill, 1 in 10 people ingest food. contaminated and 420 000 die from this same cause (OMS, 2015). Food can be contaminated along the productive chain and transport (Fatem, 2011).

ETA-causing bacteria that are listed as dangerous include enterohemorrhagic *Escherichia coli* (particularly serovar O157: H7), *Campylobacter jejuni*, and *Salmonella typhimurium* (Blackburn and McClure, 2002). According to the CDC (2017), the bacterium *Salmonella* has been found in fruits of tomato, melon, pumpkin and mango, while *E. coli* O157:H7 in romaine lettuce and organic spinach.

The objective of this research was to identify the presence of enterobacteria in various sources of inputs used in agricultural production in La Comarca Lagunera and determine the risk involved in their use in agricultural activities.

Materials and methods

Sampling

The sampling of the various samples was made in the months of August to September during the years 2014-2016, in common and agricultural areas of the municipalities of San Pedro, Francisco I. Madero and Matamoros in the state of Coahuila, and of the municipalities of Lerdo and Tlahualilo in the state of Durango (Table 1).

Table 1. Type of samples analyzed in La Comarca Lagunera and number of samples.

Type of sample	Location	N
Water from River Nazas	Common León Guzmán *	10
	Dam Francisco Zarco *	20
Water form well	Common Hidalgo **	15
	Fco. I. Madero **	20
	Common Porvenir **	15
	Common Venecia **	27

Type of sample	Location	N
Residual water	Gómez Palacio *	20
Water form canal	Common Hidalgo **	10
	Common Escuadrón **	10
	Common Hormiguero **	12
	Common Coyote **	20
	Common 20 de noviembre **	12
	Common Jaboncillo **	8
	Common Santa María **	7
	Common Presa de Guadalupe **	8
	Common Finisterre **	10
	Common Florida **	10
	Common San Felipe *	12
	Common Viñedo *	10
	Common Venecia *	20
	Common Lucero *	20
	Tlahualilo *	20
	Matamoros **	12
Soil (onion growing)	Fco. I. Madero **	27
	Matamoros **	22
	Common Hidalgo **	13
Soil (chili cultivation)	Common Porvenir **	12
	San Pedro de las Col. **	10
Soil (tomato crop)	Common Hidalgo **	20
	Common Florida **	16
	Common Venecia *	10
Bovine manure	Fco. I. Madero **	20
	Common Venecia *	20
	Common Lucero *	20
Goat manure	Common Florida **	15
	Common Rosita **	15
	Common Venecia *	20
Chicken manure	Common Florida **	10
	Common Hidalgo **	10
	Common Esfuerzo **	12
Total		600

*= located in the state of Durango; **= located in the state of Coahuila. A total of 600 samples were collected (agricultural land 130, manure 142 and water 328).

The methodology for taking, handling and transporting samples was based on NOM-230-SSA1-2002 for water samples, and NOM-004-SEMARNAT-2002 for soil and manure samples.

The samples for water consisted of 500 ml, the samples for soil of 500 g and the samples for the manure of 300 g in fresh and 500 g in dry. After being collected, the samples were immediately stored on ice at a temperature below 10 °C and transported to the laboratory where they were stored at 4 °C. The microbiological analyzes were carried out within 24 h after the collection of the samples.

Microbiological analysis

The analysis for the presence of fecal coliforms was carried out following the NOM-004-SEMARNAT-2002. In the presumptive test, 1 ml of each dilution prepared in tubes containing lactosed broth was passed and incubated at 35 °C ±0.5 °C for 24 ±2 h, + 24 h if necessary. The positive tubes of the presumptive test were reseeded by triple roasting (sterilized to the lighter and cooled) in tubes containing EC broth and incubated at 44.5 ±0.2 °C in a water bath for 24 ±2 h. The result was positive when there was gas production from the fermentation of the lactose contained in the EC medium.

The positive tubes were transferred to Mac Conkey sorbitol agar with cefixime and tellurite, incubated from 18 to 24 h at 37 °C, then transferred to TSAYE agar and EMB agar, and incubated for 24 h at 35 °C. The colonies characteristic of *E. coli* was isolated and subjected to the biochemical tests of indole (+), TSI (A/A + Gas), Citrate of Simmons (-), sorbitol (-), being sorbitol negative. Since the Enterobacteria group also includes *Klebsiella*, it was isolated in the samples analyzed with this methodology.

The isolation and identification of *Salmonella* was carried out according to the protocol described by Andrews *et al*. (2007), which briefly describes, 25 mL or 25 g of sample were passed to 225 mL of lactose broth and incubated for 24 h at 35 °C. After the incubation period, the samples were transferred to tetrathionate broth (1 mL of sample plus 10 mL of tetrathionate broth) and allowed to incubate from 18 to 24 h at 42 °C, to be later sown on XLD agar (Becton Dickinson de México).

These plates were incubated from 22 to 50 h at 35 °C. Colonies with *Salmonella* characteristics were passed to biochemical tests on triple sugar and iron agar (TSI), iron-lysine agar (LIA) and Simmons citrate agar (Becton, Dickinson and Company EE UU). The identification of the genus was carried out through the biochemical tests of LIA, TSI and Simmons' Citrate.

The microbiological analyzes were performed in the microbiology laboratory of the Faculty of Agriculture and Zootechnics, located at km 28 of the Gómez Palacio-Tlahualilo highway, Durango, in the common Venecia, Municipality of Gómez Palacio, Durango, Mexico.

Results and discussion

Analysis of water samples

56.09% (184/328) of water samples (Table 2) presented contamination with some enterobacteria of the most commonly mentioned by the literature (Brisse *et al*., 2009; Puerta-Garcia and Mateos-Rodríguez, 2010). The prevalence by microorganism isolated was as follows: *Klebsiella* spp. 46.95% (154/328), *Proteus* spp. 8.53% (28/328), *E. coli* 7.92% (26/328), *Salmonella* spp. 7.31% (24/328). In this study, *Klebsiella* was identified as the main risk factor for bacterial contamination in the water used to irrigate the different crops in the Comarca Lagunera. *Klebsiella* spp. it is a ubiquitous pathogen and together with *E. coli* and *Enterobacter* spp. they are bacteria with the capacity to cause gastroenteritis (by ingestion), respiratory infections, in the ears, skin and others (Rodríguez *et al*., 2017).

Table 2. Isolated microorganism and prevalence in four types of water samples used in the irrigation of crops in La Comarca Lagunera.

Type of sample	Location	N	Microorganism	Prevalence
Water from river Nazas	Common Leon Guzmán *	10	*Klebsiella*	10/10
	Dam Francisco Zarco *	20	*Klebsiella*	10/20
Water form well	Common Hidalgo *	15	*Proteus* and	10/15
	Fco. I. Madero *	20	*Klebsiella*	15/20
	Common Porvenir *	15	*Klebsiella*	15/15
	Common Venecia *	27	*Klebsiella*	3/27
			Salmonella	
Residual water	Gomez Palacio *	20	*E. coli* and *Klebsiella*	20/20
Water form canal	Common Hidalgo *	10	*Proteus* and	10/10
	Common Escuadron *	10	*Klebsiella*	8/10
	Common Hormiguero *	12	*Proteus* and	5/12
	Common Coyote *	20	*Klebsiella*	16/20
	Common 20 de noviembre *	12	*Salmonella*	7/12
	Common Jaboncillo *	8	*Klebsiella*	7/8
	Common Santa Maria *	7	*Salmonella*	2/7
	Common Presa de	8	*Klebsiella*	3/8
	Guadalupe *	10	*Klebsiella*	2/10
	Common Finisterre *	10	*Klebsiella*	2/10
	Common Florida *	12	*E. coli*	3/12
	Common San Felipe **	10	*Salmonella*	1/10
	Common Viñedo **	20	*Salmonella*	3/20
	Common Venecia **	20	*Salmonella*	15/20
	Common Lucero **	20	*Salmonella*	13/20
	Tlahualilo **	12	*Klebsiella*	4/12
	Matamoros *		*Klebsiella*	
			E. coli	
Total		328		184/328

*= located in the state of Durango; **= located in the state of Coahuila.

The presence of *Klebsiella* in the water samples analyzed in this work represents a risk to human health and can become a big problem in the control of infections caused by this bacterium, since *Klebsiella* strains resistant to third generation cephalosporins have been isolated in fruits and vegetables that are consumed fresh (Mesbah *et al*., 2017) and whose source of contamination has been irrigation water. Another important pathogen detected in the water samples was *Salmonella* spp. This bacterium is found on a daily basis in samples of soil, water and vegetation (Puerta-García and Mateos-Rodríguez, 2010).

Its presence in water samples from irrigation canals represents a risk, since it can pass to fruits and vegetables irrigated with this water and from there to the final consumer (Goodburn and Wallace, 2013; Nuesch-Inderbinen and Stephan, 2016). The water samples taken from the Nazas River showed a prevalence of contamination with enterobacteria of 66.66% (20/30), the samples of well water a value of 55.84% (43/77), the samples of residual water a value 100% (20/20) and the channel water samples a value of 50.24% (101/201). These values are lower than that found by Luczkiewicz *et al*. (2011), who determined a prevalence of fecal coliforms of 97% in water samples; however, they are higher than that reported by Mesbah *et al*. (2017) who found a frequency of contamination by enterobacteria equal to 9% in the irrigation water.

The presence of enterobacteria in the four types of water samples analyzed in this work, and which are used as irrigation water in the crops of La Comarca Lagunera, constitutes a risk factor, since there is evidence of contamination of food of origin agricultural irrigation water and an association of foodborne outbreaks with vegetables, juices and other contaminated products, especially minimally processed or raw consumption (De Giglio *et al*., 2017).

Analysis of soil samples

In the different types of soil (Table 3) and independently of the culture established at the time of sampling, only the bacterium *Klebsiella* spp. with a prevalence of 60% (78/130).

Table 3. Isolated microorganism and prevalence in three different soils according to the crop established at the time of sampling in La Comarca Lagunera.

Type of sample	Localidad	N	Microorganism	Prevalence
Ground (Onion growing)	Fco. I. Madero **	27	*Klebsiella*	15/27
	Matamoros **	22	*Klebsiella*	10/22
	Common Hidalgo **	13	*Klebsiella*	10/13
Ground (chili cultivation)	Common Porvenir **	12	*Klebsiella*	8/12
	San Pedro de las Col **	10	*Klebsiella*	6/10
Ground (tomato crop)	Common Hidalgo **	20	*Klebsiella*	10/20
	Common Florida **	16	*Klebsiella*	11/16
	Common Venecia *	10	*Klebsiella*	8/10
Total		130		78/130

*= located in the state of Durango; **= located in the state of Coahuila.

In the soil where onion cultivation was established, a prevalence of 56.45% (35/62) was observed, for the soil where chili culture was established, a prevalence of 63.63% (14/22) and for the soil where there was tomato cultivation a prevalence of 63% was observed (29/46). *Klebsiella* spp. it is found in the environment on a daily basis, and the soil is not the exception. Rossmann *et al*. (2012) described the incidence of *Klebsiella* spp. in 11.4% in the soil rhizosphere and also reported the presence of enterobacteria with human pathogenic potential with less prevalence such as *Salmonella* and *E. coli*. In this study, neither *Salmonella* nor *E. coli* was detected in the soil samples.

Analysis of manure samples

Regarding the enterobacteria isolated in the three types of manure (Table 4), the prevalence was 41.54% (59/142). The prevalence by type of manure was as follows: cattle 33.3% (20/60), goat 14.0% (7/50) and 100% chicken manure (32/32).

Table 4. Isolated microorganism and prevalence in three different types of manure in La Comarca Lagunera.

Type of sample	Location	N	Microorganism	Prevalence
Bovine manure	Fco. I. Madero **	20	*E. coli*	6/20
	Common Venecia *	20	*E. coli*	6/20
	Common Lucero *	20	*Klebsiella*	8/20
Goat manure	Common Florida **	15	*Salmonella* spp.	3/15
	Common Rosita **	15	*Salmonella* spp.	4/15
	Common Venecia *	20	*Salmonella* spp.	0/20
Chicken manure	Common Florida **	10	*Salmonella* spp.	10/10
	Common Hidalgo **	10	*Salmonella* spp.	10/10
	Common Esfuerzo **	12	*Salmonella* spp.	12/12
Total		142		59/142

*= located in the state of Durango; **= located in the state of Coahuila.

In bovine manure, *E. coli* and *Klebsiella* spp. were isolated, while *Salmonella* spp. was isolated only in goat and chicken manure. The above is a strong risk factor for human health, because the use of antibiotics to treat humans and animals in agriculture can lead to the selection of bacteria resistant to antibiotics that escape into the environment in manures (Durso and Cook, 2014).

Environmental isolates of enterobacteria that have acquired resistance to third generation cephalosporins (3GC) constitute a crucial threat to public health as sources of resistance for pathogenic bacterial strains that could lead to a failure in antibiotic therapy (Blaak *et al*., 2014).

The presence of residues in the housing of pigs, cows, chickens, goats has been identified as an important risk factor for *Salmonella* infection (Beloeil *et al*., 2004). *Salmonella* can survive for months in remains of dust and organic matter present in pens, equipment and ventilation systems (Rajic *et al*., 2005), being this survival marked by some factors such as serotype and climatic conditions. Therefore, it is very important to use effective cleaning and disinfection protocols in the stables.

Several studies have proven that the application of an adequate cleaning and disinfection protocol is an important measure to prevent infection by *Salmonella* within the stables (Schmidt *et al*., 2004; Hautekiet *et al*., 2008).

In the case of the three types of manure analyzed (Table 3), it is observed that *Salmonella* contamination is found in the chicken manure and not cow manure, possibly due to the fact that the region is an important dairy basin, which may indicate that in bovine stables there are good excreta management practices. The development of diagnostics for enteropathogenic bacteria, associated with horticultural products, will allow to establish more adequate control measures in the production chain by identifying the sources of contamination such as irrigation water, manure, soil with a tendency to horticultural crops, towards which directed the investigation.

The results found in this research, highlight the importance of giving manure a treatment aimed at minimizing the burden of pathogenic microorganisms before being used as fertilizer in agricultural production, since it is shown that in addition to improving soil fertility, they are also carriers of zoonotic pathogens that have the potential to cause disease in humans (Hutchison *et al*., 2005).

Conclusions

The inputs evaluated in this work and that are applied in agricultural production in La Comarca Lagunera represent a risk factor for the consumer due to the presence of enterobacteria. This, in addition to being a source of diseases transmitted by food, makes producers less competitive to sell their agricultural products. In addition, the final consumer should be informed about these microbiological health hazards and promote good housekeeping practices. It also follows that more research is needed along the fruit and vegetable production chain in order to involve more professionals in agricultural safety in actions to reduce this contamination.

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