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Phylogroups, pathotypes, biofilm formation and antimicrobial resistance of *Escherichia coli* isolates in farms and packing facilities of tomato, jalapeño pepper and cantaloupe from Northern Mexico

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ABSTRACT

The most commonly used indicator of fecal contamination in fresh produce production and packing is *Escherichia coli*. In depth analysis of the prevalence and characteristics of naturally occurring *E. coli* strains in these environments is important because it can (1) serve as an indicator of sources of fecal contamination; and (2) provide information on strain pathogenicity, persistence, and other defining characteristics such as multidrug resistance. In this study, we analyzed 341 *E. coli* strains isolated from the jalapeño pepper, tomato and cantaloupe farm environments, in Northeast Mexico. Strains were isolated from produce, farmworkers' hands, soil and water. Pathotypes, genotypes, biofilm formation and antibiotic resistance were characterized. Phylogenetic subgroups and identification of diarrheagenic *E. coli* were determined by PCR; biofilm formation was quantified using a plate-based colorimetric method. Antibiotic resistance was analyzed by the Kirby Bauer diffusion disc method. Most isolates (N = 293, 86%) belonged to phylogenetic group A. Only four isolates (1.2%) were diarrheagenic: EPEC (N = 3) and ETEC (N = 1). Antibiotic resistance to tetracycline (23.2%) and ampicillin (19.9%) was high, and only 3.5% of the strains presented resistance to > 5 antibiotics. Biofilms were produced by most strains (76%), among which 34.4% were categorized as high producers. The presence of antibiotic resistant *E. coli* strains that may contain gene markers for pathogenicity and which can form biofilms suggests potential health risks for consumers.

1. Introduction

Escherichia coli is a normal inhabitant of the digestive tract of warm blooded animals, including humans (CDC, 2014). *E. coli* is one of the dominant enteric species in human feces and it has been used as an indicator of fecal contamination for close to a century. *E. coli* is often regarded as harmless; however, there are *E. coli* groups which have acquired virulence factors and have the ability to cause diarrheal disease in healthy humans (Kaper et al., 2004). A recent systematic review reported a low prevalence of pathogenic *E. coli* on farms (range 0–1.6%) and packing facilities (0–10%) from fourteen studies testing produce for pathogens. Only in the US, at least nine documented outbreaks of pathogenic *E. coli* have been linked to the consumption of fresh produce such as lettuce, spinach and sprouts from 2010 to 2017 (CDC, 2018). Produce can become contaminated in the field or from improper sanitation, handling or processing. The use of compost, sewage contaminated water for irrigation and droppings from wild and domesticated animals and birds are all considered sources of pathogen contamination on fresh produce (Liu et al., 2013).

The most commonly used indicator of fecal contamination in fresh produce production and packing is *Escherichia coli*. In depth analysis of the prevalence and characteristics of naturally occurring *E. coli* strains in these environments is important because it can (1) serve as an indicator of sources of fecal contamination through microbial source tracking (Carlos et al., 2010); (2) identify potentially pathogenic strains; (3) provide information about antimicrobial resistance profiles that can be used to understand strain emergence and clinical treatment of disease (Boehme et al., 2004); and (4) allow us to characterize of the

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Table 1
Phylogroups of *E. coli* strains isolated along the in-field production chain of jalapeño pepper, tomato and cantaloupe.

Sample type	Number of isolates (jalapeño, tomato, cantaloupe)		Jalapeño pepper	Tomato	Cantaloupe
		%	Phylogroup (%)		
Water	Source (22, 58, 5)	24.9	**A_0 (54.5)**[a], A_1 (22.7), D_1 (22.7)	**A_0 (58.6)**, A_1 (34.5), B_1 (5.2), $B2_2$ (2)	A_0 (20), **A_1 (80)**
	Irrigation hose (9, 44, 15)	19.9	**A_0 (66.7)**, A_1 (22.2), B_1 (11.1)	**A_0 (54.5)**, A_1 (27.3), B_1 (15.9), D_1 (2.3)	**A_0 (73.3)**, B_1 (6.7), D_1 (20)
Hands	Harvest (29, 8, 15)	15.2	**A_0 (69)**, A_1 (17.2), D_1 (13.8)	**A_0 (62.5)**, A_1 (25), B_1 (12.5)	**A_0 (66.7)**, A_1 (20), B_1 (13.3)
	Distribution (14, 7, 9)	8.8	**A_0 (71.4)**, A_1 (28.6)	**A_0 (42.9)**, A_1 (14.3), B_1 (28.6), D_1 (14.3)	A_0 (22.2), **A_1 (55.6)**, B_1 (11.1), D_1 (11.1)
	Packaging (5, 0, 4)	2.6	**A_0 (100)**	ND	**A_0 (75)**, B_1 (25)
Produce	Before harvest (19, 7, 6)	9.4	A_0 (47.4), **A_1 (52.6)**	**A_0 (71.4)**, B_1 (28.6)	**A_0 (83.3)**, B_1 (16.7)
	During harvest (3, 10, 3)	4.7	**A_0 (66.7)**, A_1 (33.3)	**A_0 (60)**, A_1 (10), D_1 (30)	**A_0 (66.7)**, A_1 (33.3)
	Distribution (6, 3, 5)	4.1	**A_0 (83.3)**, B_1 (16.7)	**A_1 (100)**	A_0 (20), A_1 (20), **B_1(40)**, $B2_3$ (20)
	Packaging (1, 10, 5)	4.7	**A_0 (100)**	**A_0 (70)**, A_1 (30)	A_0 (40), **A_1 (60)**
Soil	Around produce-plant sampled (9, 7, 3)	5.6	A_0 (11.1), **A_1 (77.8)**, $B2_3$ (11.1)	**A_0 (57.1)**, A_1 (14.3), D_1 (28.6)	**A_0 (100)**
Total	(117, 140, 70 = 341)	100 A0 (58.4), A1 (27.6), B1 (7.3), B22 (0.3), B23 (0.6), D1 (5.9)	**A_0 (60.7)**, A_1 (29.1), B1(1.7), $B2_3$ (0.9), D_1 (7.7)	**A_0 (57.1)**, A_1 (27.9), B1(9.7), $B2_2$ (0.6), D_1 (4.5)	**A_0 (57.1)**, A_1 (24.3), B1(11.4), $B2_3$ (1.4), D_1 (5.7)

[a] **Bold letter**: phylogroup with the highest percentage of isolates; ND: not detected.

propensity for biofilm formation to predict environmental persistence of this organism (Balcazar et al., 2015).

For microbial source tracking, a classification system has been developed based on phylogenetic cluster characteristics of this bacterium (Carlos et al., 2010; Lee, 2011). In this classification, A, B1, B2 and D constitute the main phylogroups, and the subgroups A0, A1, B1, $B2_2$, $B2_3$, D_1 and D_2, have been proposed to increase discrimination of *E. coli* strains (Carlos et al., 2010). Recently, Clermont et al. (2013) proposed a refined classification, adding four more phylogroups: C, E, F, and *Escherichia* cryptic clade I. The strains of all phylogroups differ in phenotypic and genotypic characteristics (Carlos et al., 2010; Gordon et al., 2008) such as their sugar metabolism, antibiotic resistance profiles, growth temperature ranges, ecological niches, and the presence and/or absence of select virulence factors (Carlos et al., 2010; Gordon et al., 2008). The ability to identify phylogroups has been useful in predicting human health risks. For example, diarrheal disease-causing *E. coli* are more likely of the B1 and E phylogroups and extraintestinal infection-causing *E. coli* strains are more likely of the B2 and E phylogroups (Nowrouzian et al., 2006).

E. coli, though part of the normal intestinal biota of animals and humans, has the potential to pose human health risks though acquired pathogenic virulence factors that induce diarrhea. These diarrheagenic *E. coli* (DEC) strains are classified into six different pathogenic types also known as pathotypes that include: enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and diffuse adherent *E. coli* (DAEC) (Kaper et al., 2004; Russo and Johnson, 2000). DEC strains have been associated with outbreaks of severe disease, *i.e.*, bloody diarrhea and hemolytic uremic syndrome (HUS) as well as travelers' diarrhea in association with consumption of contaminated food and water (FDA, 2012). In Mexico for instance, the presence of EPEC, ETEC, STEC (*E. coli* producer of shiga toxin) and ETEC has been reported in ready-to-eat cooked vegetable salads (León et al., 2013), dairy, meat products, seafood, fish and prepared foods (Canizalez-Roman et al., 2013).

E. coli can also be an indicator of human health risks if strains are resistant to one or more antibiotics, and studies have identified isolates from agricultural foodstuff and vegetables in which strains were resistant to more than five antibiotics (Boehme et al., 2004; Schwaiger et al., 2011). The antibiotic resistance and susceptibility profiles of *E. coli* strains vary depending on geographic location, time of exposure to the antimicrobial compound, and other environmental factors (Dombek et al., 2000). Finally, the ability of *E. coli* to form biofilms may represent a strategy for strains to persist in produce and the production environment. Biofilms can protect bacteria from sanitizers, predation, desiccation and UV radiation (Costerton et al., 1995), providing an advantage for bacterial survival and persistence.

Mexico is one of the top producers of fresh produce and a significant trading partner with the U.S. and other countries. Eleven outbreaks have occurred in the US due to the consumption of contaminated produce originating from Mexico in the period 2006 to 2017 (CDC, 2018). The associated contaminated produce included sprouts, leafy greens, spinach and lettuce (FDA, 2012). Hence, there is a need for better understanding of the characteristics of naturally occurring *E. coli* isolates from this region of the world. The purpose of this study was to identify the phylogroups, pathotypes, antibiotic resistance profiles and biofilm formation ability of *E. coli* strains previously isolated and archived (Heredia et al., 2016) from a longitudinal study along the production chain of jalapeño pepper, tomato and cantaloupe from Nuevo Leon and Coahuila, Mexico. This information provides knowledge about the *E. coli* strains circulating in the farm environment, the possible sources of contamination in production, and the likelihood of pathogenic and antibiotic resistant strains that could provide a risk to public health.

2. Material and methods

2.1. Bacterial strains

In a previous study, 341 *E. coli* isolates (117 from jalapeño pepper farms, 154 from tomato farms and 70 from cantaloupe farms) were obtained from the production chain in the Nuevo Leon and Coahuila states in Mexico (Heredia et al., 2016). Strains were isolated from water [the intake before the irrigation hose (called source) (24.9%) and the in-field irrigation hose (19.9%)]; from farmworkers hands [during harvest (15.2%), at the distribution point (8.8%), or at packaging (2.6%)]; produce [before harvest (9.4%), during harvest (4.7%), at distribution (4.1%), and during packaging (4.7%)]; and from soil (5.6) (Table 1). Control strains included *E. coli* O157:H7 ATCC 43895 (EHEC, kindly provided by Dr. Lynne McLandsborough, University of Massachusetts, Amherst, MA, USA); *E. coli* ATCC 25922 (non-pathogenic, donated by Becton Dickinson Co., Mexico); and *E. coli* O111:NM ATCC 43887 (EPEC), *E. coli* O78:H11 ATCC 35401 (ETEC) (both commercially acquired); and *E. coli* 042 (EAEC) (donated by Dr. Fernando