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Distribution of Locus of Adhesion and Autoaggregation and *hes* Gene in STEC Strains from Countries of Latin America

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic food pathogens associated with foodborne diarrheal illness, hemorrhagic colitis, and complications such as hemolytic uremic syndrome (HUS). The ability to adhere to epithelial cells is an important virulence trait, and pathogenicity islands (PAIs) play an important role on it. Some STEC carrying a PAI named locus of enterocyte effacement (LEE-positive) have been frequently associated to HUS; however, STEC that do not carry LEE (LEE-negative) have also been associated with this outcome. The burden of disease caused by LEE-negative STEC has increased recently in several countries like Argentina, Chile, and Paraguay. A new PAI –the Locus of Adhesion and Autoagregation (LAA)—has been associated to severe disease in humans. In this study, we aimed to analyze the distribution of LAA and its possible predictor, the gene *hes*, in LEE-negative STEC strains isolated from Chile and Paraguay from different sources. The presence of the different LAA modules and *hes* were detected by PCR. LAA was found in 41.6% and 41.0% of strains isolated from Chile and Paraguay, respectively. Strains were isolated from diverse origins and belonged to several serogroups including O91, O103, and O113. The *hes* gene was detected in 50% of the isolates from Paraguay and Chile. Therefore, the detection of LAA and *hes* in STEC could complement current genetic evaluation schemes, allowing to classify LEE negative STEC strains as LAA-positive or LAA-negative STEC strains.

Introduction

Foodborne pathogens are an important public health issue in many countries and are responsible for a substantial burden of disease in the developed world. In developing countries, such as Argentina, Paraguay, and Chile, this problem is of great concern [1]. One of the microorganisms involved in foodborne diseases is Shiga toxin-producing *Escherichia coli* (STEC), a diverse group of bacteria capable of causing severe human diseases such as hemorrhagic colitis (HC) and the hemolytic-uremic syndrome (HUS) [2]. Cattle are the main reservoir of STEC; however, pigs, dairy products, nuts, seeds, water -which has increasingly become a concern as a source of contamination for fruits and vegetables-, and person to person contact have been shown to commonly participate in transmission [3–5]. STEC surveillance systems are different in each Latin American country. In general, disease incidence data relies primarily on either the foodborne disease surveillance system (Argentina and Paraguay) and/or through the acute diarrheal surveillance system (Argentina, Chile, and Paraguay, among others). Argentina has the highest number of cases caused by STEC worldwide: HUS

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incidence is 8.4 cases per 100,000 children less than 5 years old [6]. On the other hand, the incidence is 3.4 cases per 100,000 children in Chile (Metropolitan Region) [7]. In both countries HUS represents one of the main causes of acute renal failure in children with lethality ranges from 2 to 6% [8]. In Paraguay, routine surveillance is performed with all the stool cultures that are sent to the Central Public Health Laboratory. The incidence of HUS is low. Between 2013 and 2015, eight STEC diarrhea cases and ten HUS cases without STEC isolation were reported [8, 9].

STEC refers to the pathotype capable of producing Shiga toxin type 1 (Stx1), type 2 (Stx2), or both, which are encoded by *stx1* and *stx2* genes, respectively [2]. Epidemiological studies suggest that STEC strains encoding *stx2* are more virulent than those harboring *stx1* only [10]. Although Stx1 is less cytotoxic than Stx2, it may potentially cause disease in humans, but the information about its clinical implications is limited [11]. In addition, some STEC strains carry the Locus of Enterocyte Effacement (LEE), a pathogenicity island (PAI) which encodes genes necessary to produce attaching and effacing (A/E) lesions on the intestinal epithelium. LEE is also carried by enteropathogenic *E. coli* (EPEC) [12] and *Escherichia albertii* strains which might have been misidentified as LEE-positive STEC or EPEC because they carry the *eae* gene [13]. However, STEC strains lacking LEE (LEE-negative), such as those belonging to serogroups O91, O113 and O174 [14], have also been isolated from cases of severe illness, including HUS [15]. A great number of adhesins, including the pO113-encoded autoagglutinating adhesin (Saa), have been linked to the pathogenesis of LEE-negative STEC [16]. Montero et al. [17] described and characterized a member of the Heat resistant agglutinin family (Hra Family), named Hemagglutinin from Shiga toxin-producing *E. coli* (Hes) that participates in several colonization-associated phenotypes, including hemagglutination, adhesion and autoaggregation. Hes is encoded by a gene located in a PAI of 86-kb chromosomal mosaic element named the Locus of Adhesion and Autoaggregation (LAA), which contains 80 genes organized into four modules: module I (*hes* and other genes), module II (*iha, lesP* and others genes), module III (*pagC, tpsA, tpsB* and other genes) and module IV (*agn43* and other genes). Moreover, LAA may be present as a "complete" (4 modules) or "incomplete" (with less than 4 modules) structure [17].

LAA presence is associated with severe human disease and although the mechanisms used by LEE-negative STEC strains to colonize the human intestine are not clear yet [17]. Recently, the role of LAA in intestinal colonization was demonstrated in a murine model of STEC infection, suggesting that LAA may be also involved in the adherence of STEC to the human intestine [18, 19]. Moreover, the association of LAA with *stx1a*, *stx2a*, *stx2d* and *cdtB* toxin genes that cause severe disease has been demonstrated [18]. In a previous study in Argentina, our group has demonstrated the presence of LAA in 46% of LEE-negative STEC strains isolated from different sources [20]. Because the incidence of HUS caused by LEE-negative STEC strains has been increased in several countries [8], in this study, we aimed to analyze the distribution of LAA and *hes* in LEE-negative STEC strains isolated in Chile and Paraguay from different sources.

Materials and Methods

Strains and Serotyping

A total of 128 LEE-negative STEC strains were analyzed: 72 from Chile and 56 from Paraguay. STEC strains were isolated from beef cattle ($n=94$), meat ($n=31$), cheese ($n=2$) and wild bird ($n=1$). These isolates were previously analyzed for the presence of *stx1, stx2,* and *saa* genes by PCR (Table 1) [21]. The serogroup was determined by microagglutination test described by Guinée et al. [22] and modified by Blanco et al. [23] (Table 1).

PCR Amplification

STEC strains were characterized by multiplex PCR to detect LAA modules I, II, and III [17]. Additionally, the presence of *agn43*, as a marker of module IV, and *hes* were characterized by monoplex PCR [20, 24]. One LEE positive STEC strain was used as a negative control (O157:H7 EDL933). PCR reactions (multiplex and monoplex) were performed and standardized in a total volume of 50 µl by using a T-17 thermal cycler (Ivema). The reaction mixture contained 500 mM KCl, 100 mM Tris–HCl pH 9, Triton X-100, 25 mM MgCl2, 200 µM 4 deoxynucleotides (dATP, dGTP, dCTP, dTTP), 1U of TaqDNA Polymerase Highway® (Inbio), and 5 µl of DNA. The DNA was obtained by boiling bacteria suspended in sterile water for 10 min as previously described [25]. The LAA and *hes* primers were reported by Montero et al. [17]. Amplification products were separated by electrophoresis on 2% agarose gels containing 0.8 µg/ml of ethidium bromide in running buffer and were visualized in a UV transilluminator.

Results

STEC strains were considered as LAA-complete when they harbored modules I, II, III, and IV and were considered as LAA-incomplete when less than four modules were detected.

Complete LAA structure was identified in strains from Paraguay and Chile in similar frequencies: 41.0% (23/56) and 41.6% (30/72), respectively (Figs. 1 and 2). Similarly,

Table 1 Virulence profile, serogroups, and LAA presence of LEE-negative STEC strains

Origin	Serogroup (*n*)	LAA-complete	LAA-incomplete	LAA-negative	Virulence profile	Origin	Serogroup (*n*)	LAA-complete	LAA-incomplete	LAA-negative	Virulence profile
CH	O3 (1)			+	*stx1, saa*	CH	O130 (1)		+(II)		*stx2*
CH	O15 (1)			+	*stx2, saa*	CH	O153/O178 (1)		+(I and II)		*stx1, stx2, saa, hes*
CH	O22(2)			+	*stx2*	CH	O153/O178 (1)	+			*stx2, hes*
CH	O22(1)			+	*stx2, saa*	CH	O153/O178 (1)	+			*stx1, stx2, saa, hes*
CH	O22(1)	+			*stx2, hes*	CH	O153/O178 (1)	+			*stx2, saa, hes*
CH	O26 (1)	+			*stx1, saa, hes*	CH	O153/O178 (1)	+			*stx1, stx2, hes*
CH	O48 (1)	+			*stx1, stx2, hes*	CH	O168 (3)			+	*stx2, saa*
CH	O76 (1)		+(I and II)		*stx1, saa, hes*	CH	O168 (1)			+	*stx2*
CH	O76 (1)		+(II)		*stx1*	CH	O171 (2)	+			*stx2, hes*
CH	O82(1)		+(III)		*stx1, stx2, saa*	CH	O171 (1)	+			*stx2, saa, hes*
CH	O82(1)	+			*stx1 saa, hes*	CH	O171 (2)			+	*stx2, saa*
CH	O91 (3)	+			*stx2, hes*	CH	O171 (1)			+	*stx2*
CH	O91 (1)	+			*stx2, saa, hes*	CH	O174 (1)	+			*stx2, hes*
CH	O93 (4)			+	*stx2, saa*	CH	O174 (1)	+			*stx2, saa, hes*
CH	O93 (2)			+	*stx2*	CH	O174 (1)			+	*stx2*
CH	O93 (1)		+(II)		*stx2*	CH	O174 (1)		+(I and II)		*stx2, saa, hes*
CH	O98 (1)			+	*stx1, saa*	CH	O174 (1)			+	*stx2, saa*
CH	O112 (1)		+(I)		*stx1, stx2, saa, hes*	CH	O181 (1)	+			*stx2, saa, hes*
CH	O113 (2)	+			*stx2, saa,hes*	CH	O183 (1)			+	*stx1, stx2, saa*
CH	O113 (1)	+			*stx1, stx2, saa, hes*	CH	O185 (1)	+			*stx2, hes*
CH	O113 (2)	+			*stx2, saa*	CH	O185 (1)		+(I)		*stx2, hes*
CH	O113 (1)			+	*stx1, stx2, saa*	CH	O185 (1)	+			*stx2, saa,hes*
CH	O116 (3)		+(I and II)		*stx2, saa, hes*	CH	NT (1)	+			*stx1,stx2, saa, hes*
CH	O116 (2)	+			*stx2, saa, hes*	CH	NT (1)	+			*stx1, stx2, hes*
CH	O116 (1)	+			*stx2, hes*	CH	NT (2)			+	*stx2, saa*
CH	O116 (1)			+	*stx1, saa*	CH	NT (1)			+	*stx1, saa*
CH	O130 (1)			+	*stx2*	CH	NT (1)		+(II)		*stx2*
						CH	NT (1)		+(III)		*stx1, stx2, saa*
P	O22 (2)	+			*stx2, hes*	P	O115 (1)			+	*stx1, saa*
P	O22 (1)			+	*stx2, saa*	P	O115 (1)	+			*stx2, saa, hes*
P	O28 (1)			+	*stx1, stx2, saa*	P	O128 (1)	+			*stx2, saa, hes*
P	O54 (1)	+			*stx1, stx2, saa, hes*	P	O130 (1)	+			*stx1, stx2, hes*
P	O74 (1)	+			*stx1, stx2, saa, hes*	P	O130 (1)		+(III)		*stx1, stx2, saa*

Table 1 (continued)

Origin	Serogroup (*n*)	LAA-complete	LAA-incomplete	LAA-negative	Virulence profile	Origin	Serogroup (*n*)	LAA-complete	LAA-incomplete	LAA-negative	Virulence profile
P	O74 (1)		+ (I and II)		*stx2, saa, hes*	P	O141 (1)			+	*stx2, saa*
P	O76 (1)			+	*stx1, stx2*	P	O150 (1)	+			*stx1, stx2, saa, hes*
P	O83 (1)			+	*stx2, saa*	P	NT (2)	+			*stx2, saa, hes*
P	O87 (1)			+	*stx2, saa*	P	NT (6)	+			*stx1, stx2, saa, hes*
P	O88 (1)			+	*stx2*	P	NT (4)			+	*stx2, saa*
P	O91 (1)	+			*stx2, saa, hes*	P	NT (3)			+	*stx1, saa*
P	O103 (6)	+			*stx1, stx2, saa, hes*	P	NT (5)			+	*stx1, stx2, saa*
P	O103 (1)		+ (I and II)		*stx1, stx2, saa, hes*	P	NT (1)			+	*stx1, stx2*
P	O103 (1)			+	*stx1, stx2, saa*	P	NT (3)		+ (2 = I and II / 1 = I)		*stx1, stx2, saa, hes*
P	O103 (1)		+ (II)		*stx1, stx2, saa*	P	NT (1)		+ (III)		*stx1, stx2*
P	O115 (1)			+	*stx1*	P	NT (1)		+ (III)		*stx1, stx2, saa*
						P	NT (1)		+ (III)		*Stx2, saa*

(*P = Strains isolates from Paraguay/*CH = Strains isolates from Chile/ + = LAA complete/*I = Module I/*II Module II/*III = Module III/I and II = Module I and II)

the gene *hes* was detected in 50% of the isolates from Paraguay (28/56) and Chile (36/72) (Table 1). STEC strains carrying LAA (complete or incomplete) were distributed in 23 serogroups. The most frequent were O91 (4/4, 100%), O113 (5/6, 83%), O103 (6/9, 67%) and O174 (2/5, 40%) (Table 1).

Regarding toxins, the *stx2* gene was detected in 30% (7/23) and 73% (22/30) of STEC strains harboring LAA from Paraguay and Chile, respectively (Table 1).

Discussion

Although *E. coli* O157:H7 is the most prevalent serotype associated with HUS, there is growing concern on the global emergence of LEE-negative STEC that have been associated with outbreaks and/or severe human illness [26]. LEE-negative STEC strains have been detected in humans, animals, food, and the environment. Still, the pathogenic mechanisms used by this group of strains to colonize humans are yet to be elucidated [27]. LAA is a PAI of an emerging group of STEC strains that cause severe diseases in humans. In fact, the complete LAA island is present in LEE-negative STEC strains which have been isolated from cases of hemorrhagic colitis and HUS [17].

In this study, the complete LAA was widely distributed in STEC strains from different origins and belonging to several serogroups. LEE-negative STEC strains isolated from Chile (41.6%) and Paraguay (41.0%) harbored the complete LAA island with a frequency of detection that is similar to that previously reported Argentina (46%) [20].

Stx2-producing strains are more often associated with HUS than strains that produce Stx1 [28]. In this study, we detected *stx2* in 30% and 73% of the isolated LAA positive obtained from Paraguay and Chile, respectively. Likewise, Colello et al. [20] found a significant association between the presence of a complete LAA island and *stx2* in strains isolated in Argentina. In addition, LEE-negative STEC strains of serogroups O91, O113 and O174 have been isolated from cases of severe illness [29], and in our study most of the isolates belonging to these serogroups harbored LAA. PAIs like LAA has had a remarkable role in the emergence of LEE-negative STEC strains and may contribute to the evolution and virulence of pathogenic *E. coli* [18].

Montero et al. [17] suggested that *hes* is a potential genetic marker for LAA, and this raises a new possible epidemiological scenario [17] for STEC since it is widely distributed in LEE-negative STEC strains. Our results revealed that *hes* was present in all LAA positive strains, carrying the complete island. In concordance with Colello et al. [20], we also observed that *hes* was one of the most prevalent genes in LEE-negative STEC strains [18, 20].

Therefore, our work provides new data about the presence of LAA and the *hes* gene in STEC strains from different

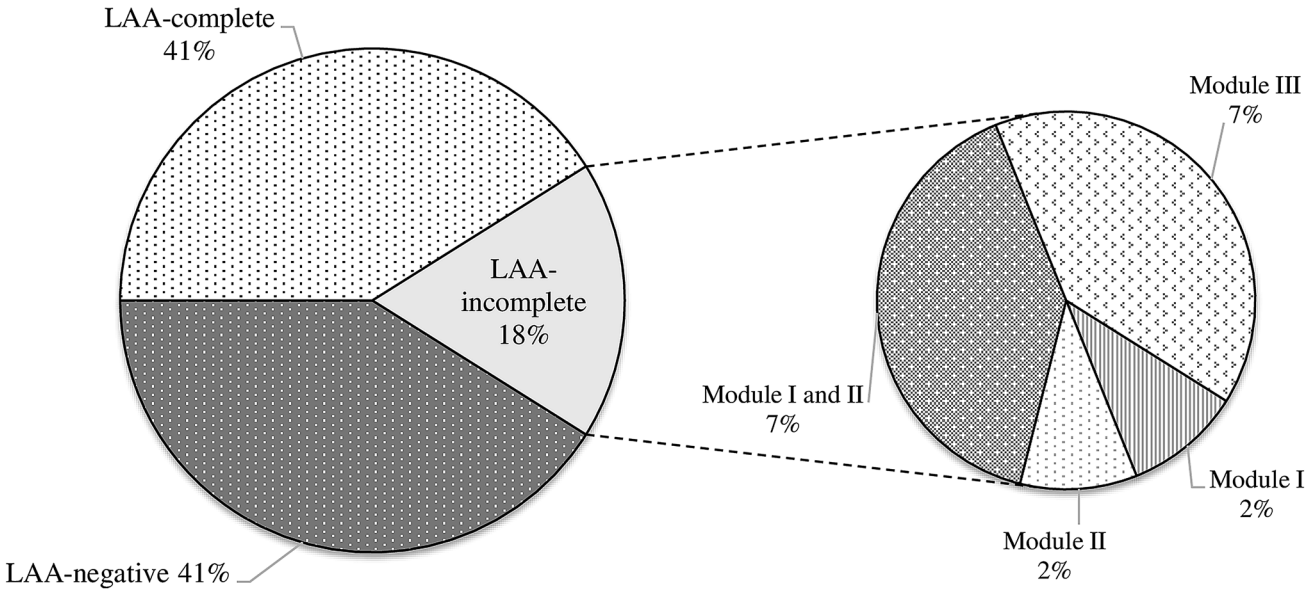

Fig. 1 Distribution of LAA detection in STEC strains isolated at different origins from Paraguay

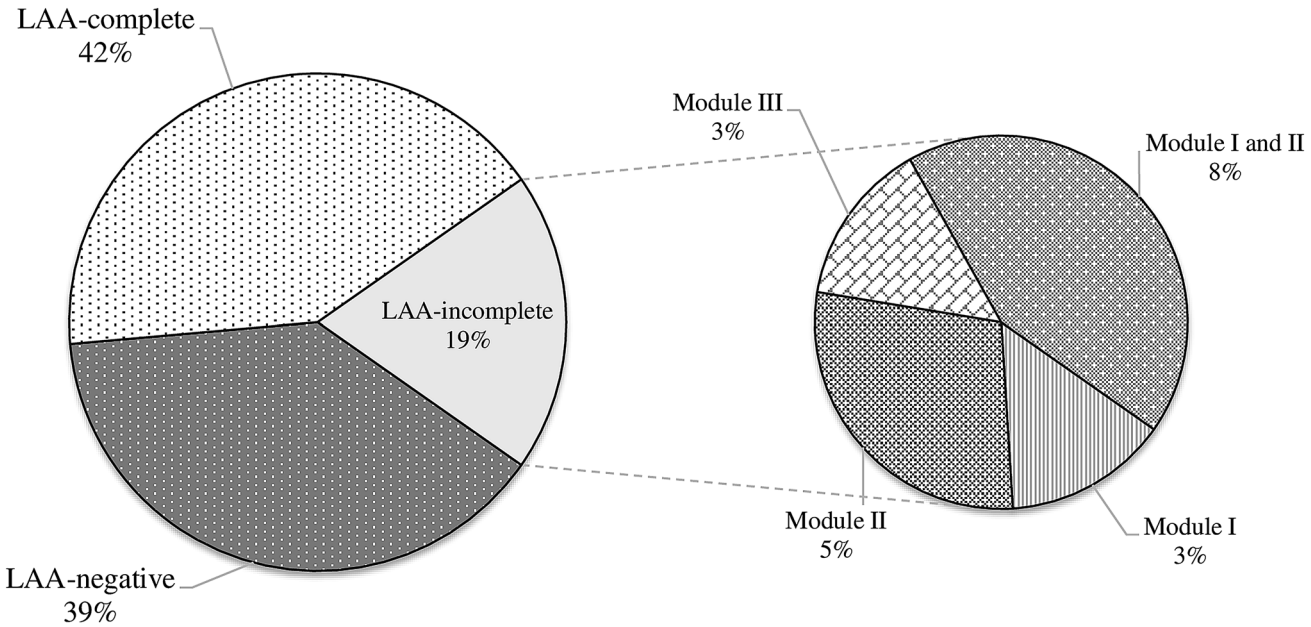

Fig. 2 Distribution of LAA detection in STEC strains isolated at different origins from Chile

sources and isolated in two Latin-American countries. These results support previous findings that suggest that the current STEC classification based on the presence of LEE might be insufficient to detect human pathogenic STEC strains, and that *hes* detection could complement current genetic evaluation schemes to detect strains representing risk [17, 30].

Concluding, to define a STEC strain as pathogenic is complex because there are not combinations of markers that can predict the potential of a STEC strain to cause human disease. In LEE positive strains, *stx2* and *eae* are predictive markers for severe disease. In LEE-negative STEC strains, the scenario is even more complex because there are not additional virulence factors to *stx2* associated with severe disease. In this context, *hes* could be used as a marker allowing classify the LEE negative STEC strains

as LAA positive or negative STEC strains, and therefore, inferring the potential risk of LEE-negative strains.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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