Rev Argent Microbiol. 2016;xxx(xx):xxx–xxx

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

BRIEF REPORT

Serotypes, virulence profiles and *stx* subtypes of Shigatoxigenic *Escherichia coli* isolated from chicken derived products

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Received 16 December 2015; accepted 12 April 2016

KEYWORDS
STEC;
Chicken;
Serotypes;
Virulence factors;
stx subtypes

Abstract Shigatoxigenic *Escherichia coli* (STEC) is a foodborne pathogen that causes hemolytic uremic syndrome (HUS) and the consumption of chicken products has been related to some HUS cases. We performed a non-selective isolation and characterization of STEC strains from retail chicken products. STEC isolates were characterized according to the presence of stx_1, stx_2, *eae*, *saa* and *ehxA*; *stx* subtypes and serotypes. Most of them carried stx_2, showing subtypes associated with severe human disease. Although reported in other avian species, the stx_{2f} subtype was not detected. The isolates corresponded to different serotypes and some of them, such as O22:H8, O113:H21, O130:H11, O171:H2 and O178:H19, have also been identified among STEC isolated from patients suffering from diarrhea, hemorrhagic colitis, HUS, as well as from cattle. Considering the virulence profiles and serotypes identified, our results indicate that raw chicken products, especially hamburgers sold at butcheries, can be vehicles for high-risk STEC strains.

PALABRAS CLAVE
STEC;
Pollo;
Serotipos;
Factores de virulencia;
Subtipos de *stx*

Serotipos, perfiles de virulencia y subtipos de *stx* en *Escherichia coli* productor de toxina Shiga aislados de productos de pollo

Resumen *Escherichia coli* productor de toxina de Shiga (STEC) es un patógeno transmitido por alimentos que causa el síndrome urémico hemolítico (SUH). Algunos casos de SUH están relacionados con el consumo de productos de pollo. Se realizó el aislamiento no selectivo y la caracterización de cepas STEC provenientes de productos de pollo atendiendo a la presencia de stx_1, stx_2, *eae*, *saa* y *ehxA*, subtipos de *stx* y serotipos. La mayoría de los aislamientos

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http://dx.doi.org/10.1016/j.ram.2016.04.009

Please cite this article in press as: Alonso MZ, et al. Serotypes, virulence profiles and *stx* subtypes of Shigatoxigenic *Escherichia coli* isolated from chicken derived products. Rev Argent Microbiol. 2016. http://dx.doi.org/10.1016/j.ram.2016.04.009

portaba stx_2 y subtipos de *stx* asociados con enfermedades graves en humanos. Aunque se ha detectado en otras especies aviares, el subtipo stx_{2f} no se encontró. Se detectaron diferentes serotipos, entre ellos O22:H8, O113:H21, O130:H11, O171:H2 y O178:H19, también identificados como STEC aislados de pacientes con diarrea, colitis hemorrágica y SUH, y de ganado bovino. Teniendo en cuenta los perfiles de virulencia y los serotipos identificados, nuestros resultados indican que los productos de pollo crudos, especialmente las hamburguesas que se venden en las carnicerías, pueden ser vehículos de cepas STEC de alto riesgo.

Shigatoxigenic *Escherichia coli* (STEC) is a foodborne pathogen of public health importance that causes diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in humans. The main virulence factors of STEC are Shiga toxins (Stx1 and Stx2), which inhibit protein synthesis by inactivating ribosome function[8]. The Stx1 group is more homogenous than Stx2 since it includes only three subtypes. In contrast, a great number of subtypes have been identified for Stx2. The *stx* subtypes have been differently associated with HUS[6]. In addition to Shiga toxins, STEC can synthesize the adhesin intimin (encoded by *eae*), an enterohemolysin (EhxA), and an autoagglutinating protein (Saa) in some *eae*-negative strains, among other virulence factors[8].

Different STEC serogroups have been identified in strains isolated from humans suffering from gastrointestinal disease. Five STEC serogroups (O26, O103, O111, O145, O157) are considered to be the ''top five'' serogroups most frequently associated with severe human disease in the European Union, and two others (O45 and O121) are also regarded as the most pathogenic ones in the USA. The serotype most frequently associated with outbreaks and sporadic cases of severe disease is O157:H7; however, more than 50% of all STEC infections are attributed to non-O157 strains[3].

STEC transmission occurs through the consumption of contaminated food or water, direct contact with animals or their environments, and person-person contact[8]. With regard to food, the consumption of chicken products has been related to HUS cases, but most of the studies performed on this kind of products have been focused only on the detection of STEC O157:H7[2,13]. Therefore, the aim of this study was to perform a non-selective isolation and characterization of STEC strains from retail chicken products.

Samples analyzed in the present study corresponded to 10 giblets and 54 chicken hamburgers previously identified as *stx*-positive by Alonso et al.[1] in a screening of 300 giblets and 300 chicken hamburgers. Peptone water cultures were stored at −70 °C with 20% (v/v) glycerol. To isolate the STEC strains, an aliquot of each *stx*-positive culture, was streaked on MacConkey agar plates and incubated at 37 °C for 24 h[11]. Individual colonies were analyzed by a multiplex PCR to detect stx_1, stx_2, *eae*, *saa* and *ehxA* genes with the PCR protocol and primers described by Paton and Paton[12]. Amplification products were electrophoresed in 2% agarose gels and stained with ethidium bromide. Only one colony was further characterized except when colonies with different virulence profiles were detected by this multiplex PCR.

As several samples were contaminated with *Proteus*, subsequent cultures were streaked repeatedly on cysteine lactose electrolyte deficient agar (CLED) to obtain pure colonies of *E. coli*. Afterwards, the absence of *Proteus* was verified by culture on a non-selective medium such as trypticase soy agar (TSA).

The O-antigens were determined by the microagglutination technique, and H antigens were determined by the tube agglutination technique using antisera provided by the Laboratorio de Referencia de *E. coli* (LREC) (Lugo, Spain) as described by Fernández et al.[5].

To subtype stx_1 and stx_2 genes, PCR-restriction fragment length polymorphism (RFLP) assays were used[9]. In addition, a monoplex PCR described by Schmidt et al.[14] was used to detect the stx_{2f} subtype.

Twenty-three STEC isolates were recovered from 54 *stx*-positive cultures of chicken hamburgers and only one isolate was obtained from 10 *stx*-positive giblet samples (Table 1). It was not possible to obtain any STEC isolate from some *stx*-positive samples although up to 200 colonies from those samples were analyzed.

Isolates carrying only the stx_2 gene predominated over the strains carrying both stx_1 and stx_2 or only stx_1, a similar trend to studies from other countries that detected stx_2 and not stx_1 in STEC isolated from chicken meat. This finding is important considering that Stx2 is more cytotoxic than Stx1[8], and is associated with high virulence in humans[6].

None of the STEC isolates carried the *eae* gene but some of them harbored the *saa* gene. STEC isolates that were *saa*-positive and *eae*-negative, belonging to serotypes O91:H21 and O113:H21 have been isolated from human patients with HUS[8]. Noticeably, in the present study O113:H21 isolates positive for *saa* were found in 3 samples and also harbored the $stx_{2EDL933}$ subtype which has been associated with severe human disease.

Five virulence profiles could be determined by the multiplex PCR described by Paton and Paton[12], with stx_2 being the predominant profile (62.5%), followed by stx_2 *ehxA saa* and stx_1 stx_2 *ehxA saa* (17 and 12.5%, respectively). Furthermore, when the *stx* subtypes were also considered, 9 virulence profiles could be determined (Table 1).

With regard to STEC from chicken and derived products, there are few studies which identified the *stx* subtypes, and furthermore, these studies were focused exclusively on the characterization of O157:H7 strains[2]. In the present study, all stx_1-positive isolates possessed the $stx_{1EDL933}$ subtype, which has been associated with HUS cases and predominates in stx_1-positive isolates from cattle and meat products[9]. As

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