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BRIEF REPORT

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

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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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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

Table 1 Serotypes and virulence genes of STEC isolated from chicken products.

Number of isolates	Sample	Origin	Serotype	Virulence genes
1	Hamburger	Butchery	O22:H8	$stx_{2EDL933}$
1	Hamburger	Butchery	O91:H14	$stx_{1EDL933}$ *ehxA*
1	Hamburger	Butchery	O91:H40	$stx_{2EDL933}$ *ehxA*
3	Hamburger	Butchery	O113:H21[a,b]	$stx_{2EDL933}$ *ehxA saa*
2	Hamburger	Butchery	O117:H7	stx_{2vhb}
2	Hamburger	Butchery	O130:H11[b]	$stx_{1EDL933}$ stx_{2vhb} *ehxA saa*
1	Hamburger	Butchery	O130:H11	$stx_{1EDL933}$ $stx_{2EDL933}$ *ehxA saa*
1	Hamburger	Butchery	O153:H28	stx_{2vhb}
1	Hamburger	Butchery	O160:H40	$stx_{2EDL933}$
1	Hamburger	Butchery	O171:H2	stx_{2O118}
1	Hamburger	Butchery	O171:H2[a]	stx_{2vha}
1	Hamburger	Butchery	O178:H19	stx_{2vha}
1	Hamburger	Butchery	ONT:H2	stx_{2O118}
1	Giblet	Butchery	ONT:H8	$stx_{2EDL933}$ *ehxA saa*
2	Hamburger	Butchery	ONT:H40	stx_{2vhb}
1	Hamburger	Poultry shop	ONT:H-	stx_{2O118}
3	Hamburger	Butchery	ONT:H-	stx_{2vhb}

[a] One O113:H21 isolate and one O171:H2 isolate were obtained from the same sample.
[b] One O113:H21 isolate and one O130:H11 isolate were obtained from the same sample.

far as we know, this is the first report about stx_1 subtypes in chicken samples. For the stx_2 gene, different subtypes were detected, but no more than one stx_2 subtype was identified within the same isolate. The stx_{2vhb} subtype was the most prevalent, accounting for 43.5% of the isolates, followed by $stx_{2EDL933}$ (8 isolates, 33%), stx_{2O118} (3 isolates, 12%) and stx_{2vha} (2 isolates, 8%). This data is important because stx_{2vhb}, $stx_{2EDL933}$ and stx_{2vha} have been frequently associated with HUS cases[6]. The stx_{2O118} subtype predominates in STEC strains from sheep, is rarely found in cattle, and, in contrast to our results, it is usually found associated with other *stx* subtypes in strains isolated from cattle and foods[9].

Although stx_{2f}-positive strains have been isolated from avian species (pigeons), and Etoh et al.[4] reported the isolation of a STEC strain harboring this subtype from a patient that had eaten raw chicken, we did not detect this subtype in any of the chicken samples.

STEC isolates belonging to O157:H7 were not detected in any of the samples, in agreement with the results obtained by other researchers who did not find this serotype in raw chicken meat and carcasses, even though they used selective methods for the isolation[15]. Indeed, there are only few studies that report the presence of STEC O157:H7 in chicken meat[2,13].

Several non-O157 serotypes were isolated from chicken products in the present study (Table 1). Some of the serotypes, such as O22:H8, O113:H21, O130:H11, O171:H2 and O178:H19, have also been isolated from patients suffering from diarrhea, HC or HUS, highlighting the importance of these findings[7]. Two hamburgers presented STEC isolates belonging to two different serotypes (O113:H21 and O171:H2 in one sample, and O113:H21 and O130:H11 in the other).

A comparison was made between STEC isolated in the present study and STEC isolates from cattle and derived products in Argentina. Noticeably, some serotypes such as O91:H14, O117:H7, O113:H21, O130:H11, O171:H2 and O178:H19 were present in both groups of strains, and also stx_2 predominated over stx_1[5,10,11]. Furthermore, some of the isolates belonging to serotypes such as O113:H21, O117:H7, O171:H2 and O178:H19 harbored the same virulence genotype and *stx* subtype as the isolates obtained from cattle, ground beef and evisceration tray samples in other studies[9,10].

In conclusion, the characterization of the STEC isolates in terms to serotype, virulence profile and *stx* subtype performed in the present study shows that chicken hamburgers can carry STEC strains that are potentially pathogenic to humans. Moreover, most of the isolates obtained from hamburgers presented the same serotype and genotype as the STEC strains recovered from cattle and derived meat products in our country.

Ethical responsibilities

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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