Accepted Manuscript

Characterisation, antimicrobial resistance and diversity of atypical EPEC and STEC isolated from COW'S milk, cheese and dairy cattle farm environments

Edson A. Rios, Jesús Santos, Isidro García-Meniño, Saskia C. Flament-Simon, Jorge Blanco, María-Luisa García-López, Andrés Otero, Jose M. Rodríguez-Calleja

PII: S0023-6438(19)30257-9

DOI: https://doi.org/10.1016/j.lwt.2019.03.062

Reference: YFSTL 7960

To appear in: *LWT - Food Science and Technology*

Received Date: 25 October 2018

Revised Date: 14 March 2019

Accepted Date: 21 March 2019

Please cite this article as: Rios, E.A., Santos, Jesú., García-Meniño, I., Flament-Simon, S.C., Blanco, J., García-López, Marí.-Luisa., Otero, André., Rodríguez-Calleja, J.M., Characterisation, antimicrobial resistance and diversity of atypical EPEC and STEC isolated from COW'S milk, cheese and dairy cattle farm environments, *LWT - Food Science and Technology* (2019), doi: https://doi.org/10.1016/j.lwt.2019.03.062.

CHARACTERISATION, ANTIMICROBIAL RESISTANCE AND DIVERSITY OF ATYPICAL EPEC AND STEC ISOLATED FROM COW'S MILK, CHEESE AND DAIRY CATTLE FARM ENVIRONMENTS

Rios, Edson A.[a]; Santos, Jesús[b]; García-Meniño, Isidro[c]; Flament-Simon, Saskia C[c]; Blanco, Jorge[c]; García-López, María-Luisa[b]; Otero, Andrés[b]; Rodríguez-Calleja, Jose M.[b,*]

[a]Department of Preventive Veterinary Medicine, Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil

[b]Department of Food Hygiene and Food Technology, Veterinary Faculty, University of León, Spain

[c]Laboratorio de Referencia de *E. coli* (LREC), Department of Microbiology and Parasitology, Veterinary Faculty, University of Santiago de Compostela (USC), Lugo, Spain

*Corresponding author:

Jose Mª Rodríguez Calleja

Email: jm.rcalleja@unileon.es

Phone: +34 987 293 454

Fax: +34 987 291 284

ABSTRACT

This study was carried out to determine the occurrence and characteristics of enteropathogenic *Escherichia coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) strains in cow's milk, cheese and dairy cattle farm environments, and to estimate distribution of antimicrobial resistance. A collection of 18 atypical EPEC -aEPEC, 14 STEC, and one *E. albertii* was obtained and characterized from 502 samples. Occurrence of aEPEC in cow's milk was high (>6%) whereas non-O157 STEC was isolated in ca. 2% of milk samples. Detection of these diarrheagenic *E. coli* was

absent in more than 100 cheese samples obtained from raw milk. This is the first report identifying *E. albertii* (O69:HNM) in a dairy cattle farm. Nearly one-third of aEPEC strains showed antimicrobial resistance, mostly presenting a multidrug resistance pattern. One clonal complex (ST20 Cplx) containing aEPEC strains from milk and faecal samples was determined. Two STEC strains belonged to serotypes with importance in human disease (O91:H21 and O55:H8) and were isolated from air samples which suggests a high dissemination potential. Spanish bulk tank cow's milk can constitute an important source of aEPEC strains besides STEC, bearing multiple antimicrobial resistance and with high diversity of both serotypes and genetic features linked to potential human infection**.**

KEYWORDS

multidrug resistance; serotypes; diarrheagenic *E. coli*; *E. albertii; aEPEC.*

1. Introduction

Enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) are pathogenic groups of *E. coli* causing intestinal diseases and thus categorized as diarrheagenic *E. coli* (DEC). Outbreaks caused by pathogenic *E. coli* have been reported in scientific literature associated with milk and dairy products, such as cheese manufactured from raw milk (Canizalez-Roman, Gonzalez-Nuñez, Vidal, Flores-Villaseñor, & León-Sicairos, 2013; De Buyser, Dufour, Maire, & Lafarge, 2001; Verraes et al., 2015).

Typical EPEC (tEPEC) strains are characterized by the presence of the EPEC adherence factor (pEAF) plasmid which encodes the bundle-forming pili (BFP), while atypical EPEC (aEPEC) do not possess this pEAF. aEPEC strains are considered emerging enteropathogens detected worldwide as reviewed by Hernandes et al. (2009). Whereas the main reservoir of tEPEC are humans, aEPEC strains have been isolated from animal species, environment, and food samples, some of which belong to serogroups implicated in human diseases. Data reporting prevalence of

aEPEC in samples of cow's milk or cheese manufactured from cow's milk are scarce and diverse (Altalhi & Hassan, 2009; Gonzalez, Rosa, Andrade, & Tibana, 2000; Ombarak et al., 2016).

Members of STEC group are *E. coli* producing Stx1 and/or Stx2 toxins and, apart from the high virulent O157:H7 serotype, other non-O157 serogroups are considered of increasing concern for public health (Farrokh et al., 2013). As the majority of studies were focusing on O157:H7 in milk and dairy products, non-O157 STEC impact on food has not been routinely tested and thus problems associated with STEC group may have been underestimated. However, scientific reports highlight the clinical importance of non-O157 serotypes as a cause of hemolytic-uremic syndrome (HUS), whose importance in Europe and USA has increased (Johnson & Tyler, 1993; Smith, Fratamico, & Gunther, 2014; Valilis, Ramsey, Sidiq, & DuPont, 2018).

The increase in antibiotic-resistant bacteria is a serious concern all over the world and particularly in Europe (EFSA & ECDC, 2017). The global increase of multidrug-resistant *E. coli* is a threat for public health. Among the resistance mechanisms (EUCAST, 2013), an emerging one in multidrug-resistant *E. coli* is based on the production of extended-spectrum β-lactamases (ESBLs). As ESBL-producing *E. coli* isolates have been detected in food products, mainly in meat products and much less studied in milk and dairy products, health institutions are worried about their potential spreading from the food chain to humans (EFSA & ECDC, 2017; EFSA Panel on Biological Hazards, 2011).

This study was undertaken to determine the occurrence and characteristics of EPEC and STEC strains in cow's milk, cheese manufactured from raw milk and dairy cattle farm environments in Northwest Spain, and to estimate the potential of these sources acting as vehicles of AMR.

2. Material and methods

2.1. Sample collection and processing

A total of 502 samples were obtained, during the winter and summer season, from cow's milk (n=214), cheese made from raw cow's milk (n=216), and the environment of dairy cattle farms (n=72).

Samples of 60 ml of bulk tank cow's milk, obtained from 107 dairy cattle farms and located in Northwest Spain (Region of "Castilla y León"), were collected. Cheese, manufactured from raw milk and ripened during 3 month, was collected from a local cheesery. In addition, environmental samples were gathered from five farms which were chosen among those determined as positive for the presence of EPEC and/or STEC. Samples from air (n=10), water (n=15), feed (n=15), and faeces (n=15) were collected following the procedure described by Otero et al. (2013). On each dairy farm, hands of farm handlers (n=10) were sampled by a common swabbing technique and milk filters (n=7) of milking machine were aseptically introduced in sterile pouches. All samples were processed within two hours.

Each sample was processed as follows: (a) 50 ml of milk were cultured in 450 ml of Tryptone Soya Broth plus 0.6% yeast extract (TSBYE; Oxoid); (b) 25 g of cheese were homogenized in 225 ml of TSBYE in a Masticator blender (IUL SA, Barcelona, Spain); (c) airborne particles on SMAC Agar plates were directly incubated; (d) water samples of 250 ml were passed through sterile 0.45 µm filters which were incubated in 50 ml TSBYE; (e) 25 g of feed pellets was blended with 225 ml of TSBYE; (f) wet swab from handler' hands was transferred into a flask with 225 ml TSBYE; (g) milk-filter microbiota was removed by washing off with 250 ml of TSBYE; (h) two boot swabs per farm were placed in 400 ml of TSBYE. All cultures were incubated during 18 h at 37 ºC.

2.2. Isolation and characterization of strains

From an aliquot (1 ml) of each enriched broth, DNA was extracted by a boiling procedure and PCR was carried out for the presence of the target genes *stx*1, *stx*2, and *eae* using the primers and conditions reported elsewhere (Olsen et al., 1995; A W Paton & Paton, 1998; Pollard, Johnson, Lior, Tyler, & Rozee, 1990).

Presumptive positive-sample enrichments for any of the investigated genes were spread onto SMAC agar plates. After incubation (37 ºC/24h), up to 20 colonies were randomly picked up and pooled for subsequent screening by PCR for *stx*1, *stx*2, and *eae* genes as indicated above. Colonies from PCR-positive pools were individually investigated in order to isolate EPEC and/or STEC strains.

All isolates were serotyped in the Reference Laboratory for *E. coli* (LREC; University of Santiago de Compostela, Lugo, Spain) using the method previously described by Guinée et al. (1981) with all the available O (O1 to O181) and H antisera (H1 to H56).

The phylogenetic groups were determined by the quadruplex method (Clermont, Christenson, Denamur, & Gordon, 2013). Isolates that could not be assigned to any phylo-group were further investigated by PCR for identification as *E. coli*, *E. albertii* or *E. fergusonii* (Lindsey, Garcia-Toledo, Fasulo, Gladney, & Strockbine, 2017).

Amplification of *bfpA* gene for classification of EPEC isolates was performed as described earlier (Gunzburg, Tornieporth, & Riley, 1995). Strains were also studied for presence of intimin variants (Blanco et al., 2004b). TTSS (Type III Secretion System) structural and translocator-proteins (*espA*, *espB*,) and TTSS effector protein (*tir*), and their variants α, β and γ respectively, were also tested (China, Goffaux, Pirson, & Mainil, 1999). Enterohaemolysin gene –*ehlyA* was also considered (Wang, Clark, & Rodgers, 2002).

All the STEC strains were additionally characterised by PCR using conditions described previously for the following genes: subtypes of *stx* genes (Scheutz et al., 2012), e*hlyA* (Wang et al., 2002), *subAB* (Adrienne W Paton, Srimanote, Talbot, Wang, & Paton, 2004), *saa* (Adrienne W Paton & Paton, 2002), and *tia* (Tozzoli et al., 2010).

2.3. Determination of antimicrobial susceptibility

EPEC and STEC isolates were tested for susceptibility to 22 antimicrobials by the Disk Diffusion Method on Mueller Hinton Agar (Oxoid) in accordance with the standard procedure M100-S of the Clinical and Laboratory Standards Institute -CLSI (2016) and the antimicrobial

recommendation of the *European Committee on Antimicrobial Susceptibility Testing* (EUCAST, 2015).

A double disk synergy test (DDST) was performed to identified ESBL-producing isolates according to EUCAST protocol (EUCAST, 2013) as long as a PCR method to determine the ESBL-encoding genes *bla*CTX-M (Pagani et al., 2003), *bla*SHV and *bla*TEM (Monstein et al., 2007).

2.4. PFGE and MLST analysis

PulseNet International Genomic protocol for non-O157 STEC (http://pulsenetinternational.org/) was carried out for bacterial DNA analysis by PFGE in a CHEF-DRIII apparatus (Bio-Rad, Hercules, CA, USA) as described earlier (Otero et al., 2013). Multilocus sequence typing was performed following the Achtman seven-locus scheme in accordance with the conditions described elsewhere (Denamur, Clermont, & Gordon, 2015; Wirth et al., 2006). PCR product purifications, sequencing, sequence analysis, determination of clonal complexes, and a phylogenetic tree (concatenated sequences) were carried out according to Otero et al. (2013). Each gene *locus* was assigned an allele number and a sequence type (ST) was determined for each isolate in accordance with the scheme available at http://enterobase.warwick.ac.uk/species/index/ecoli.

2.5. Statistical analysis

Relationship between positive samples for STEC or EPEC and season were determined by a chi-square test of association with the software IBM SPSS Statistics for Windows v 24.0 (IBM Corp., Armonk, NY, USA.).

3. Results and discussion

3.1. Occurrence of EPEC and STEC

Data about the isolation of DEC strains according to sample origin and season are shown in Table 1. Isolates which were *stx*-/*eae*+/*bfp*A- was considered as aEPEC. They were obtained from 13 cow's milk samples (6.1 %) and six environmental samples (8.3%). Regarding STEC, our results yielded 2.3% of positive cow's milk samples (5/214) and 9.7% of positive environmental samples

(7/72) from which 14 STEC were isolated. Overall, 66.7% of positive samples for EPEC and 58.3% of samples STEC+ were obtained in summer but this seasonal relationship was not significant ($p \geq 0.05$).

STEC prevalence in cow's milk (2.3%) is in agreement with data reported in the EU by EFSA (2016) in 2015 (1.8%), 2014 (3.6%) and 2013 (2.3%). Compared with STEC occurrence, we found a higher prevalence of aEPEC (6.1%) in the milk samples studied. Retail raw milk showed 0.9% of positive samples for aEPEC in Egypt (Ombarak et al., 2016), percentage much lesser than that found in our study maybe due to the sample origin, ours being collected from bulk tanks in dairy farms. In comparison with milk from other ruminants, our data appear to suggest a clear difference as , in Spain, atypical EPEC accounted for 14.7% of ewe's milk (Otero et al., 2013) or 10.3% of goat's milk samples (Álvarez-Suárez et al., 2016).

No cheese (n=216) manufactured from raw milk was positive for STEC or EPEC. In contrast, most of studies focused on cheese in Europe ($n \leq 100$) showed STEC (Farrokh et al., 2013). Both intrinsic and extrinsic factors of the cheesemaking process, mainly pH, food additives, NaCl content, a_w value, antimicrobial interaction and/or ripening duration, play an important role on the microbial control in cheese, and probably on avoiding growth and survival of EPEC and STEC.

Milk filter, air, water and handler samples were recorded as negative for isolation of EPEC but 33.3% of faecal samples and 6.7% of feed samples were positive (Table 1). The occurrence determined in cow faeces (33.3%) is much greater than data stated in several studies on healthy cattles (around 8%) or even in faeces from diarrheic animals (ranging 12-27%) as reported elsewhere (Aidar-Ugrinovich et al., 2007; Orden et al., 2002).

STEC strains were isolated from handlers (20%), air (20%), cow-faecal samples (13.3%), and feed (6,7%). Despite the limited number of analyzed samples from environment (n=72), our results suggest that air and handlers may be vehicles for transmission of STEC within dairy farms. Occurrence of positive samples from cow faeces (13.3%) seems to fit the overall prevalence rates in Spain (Mora et al., 2011). On the other hand, feed is not considered an important contamination

route of STEC as was also revealed by our data. In contrast, no STEC was isolated from milk filter and water samples (Table 1).

3.2. Characterisation of isolates

Genetic profile and phylogenetic group in accordance with serotypes of the 33 isolates are showed in Table 2.

Phylogenetic grouping of the 18 aEPEC isolates (*eae*+/*stx*-/*bfp*A-) showed that 17 (94.4%) belonged to phylogenetic group B1, among which 13 isolates were obtained from cow's milk. All tested strains were found to have more than one of the examined virulence factors. The intimin β1 was determined in six (33.3%) milk aEPEC isolates which did not belong to classical serotypes. This intimin type is the most common among human strains of EPEC and could be frequently isolated in cow's milk as our results would point out.

Eleven out of 14 STEC isolates (78.6%) were phylogenetically grouped in group A, obtained from milk, handler, and faecal samples, and all but three were *stx*1+. The remaining three STEC isolates, obtained from air and feed samples, belonged to the phylogenetic group B1 and harboured virulence factors besides Shiga-toxin genes (Table 2). *Saa* gene was absent in STEC isolates.

The eighteen aEPEC were classified into ten different serotypes. The most frequent serotype was O156:H8 grouping five strains (27,8%), followed by O25:H2 (16.7%), O15:H2 (11.1%), and O4:H2 (11.1%). The predominant O156:H8 was detected among aEPEC strains isolated from milk samples widely distributed in different dairy cattle farms. It must be noted that this serotype is included neither in the major EPEC O-serogroups recognized by the WHO nor those narrowly linked to EPEC isolates from milk (Barkalita et al., 2016). In addition, we identified four aEPEC serogroups from faeces and milk (O25:H8, O96:H7, O109:H25, and O109:HNM) that are very uncommon in food. It is also remarkable that 3 serotype-intimin combinations (O15:H2 eae-β1, O25:H2 eae-β1, and O109:HNM eae-γ2) detected in the present study have been previously found in aEPEC isolated from human patients in Spain (Miguel Blanco et al., 2006).

Strain H8C5 *eae*+/*stx*-/*bpf*- was identified as *E. albertii* and was not associated with any phylo-group (Table 3). It belonged to serotype O69:HNM which has not been previously reported in *E. albertii* or even in EPEC strains. Moreover, to our knowledge, this species has not been previously identified in dairy cattle farms. *E. albertii* is an emerging pathogen producing gastroenteritis in human (Huys, Cnockaert, Janda, & Swings, 2003) and is mistakenly identified as EPEC.

Nine serotypes were detected in STEC strains, with six strains (42.9%), phylo-group A and *stx*1+, belonging to O140:H32 which was the only one that grouped strains from different sources (handler, milk, and faecal samples). STEC O140:H32 has been rarely reported in scientific literature (Pradel et al., 2000), with isolates commonly belonging to phylogenetic group A and carrying *stx1*. Other identified serotypes, such as O130:H21 and O3:HNM, are also uncommon in STEC strains. The strain AR10C2 was assigned to O91:H21 serotype, with clinical significance and associated with severe human disease. The strain AR6C2 belonged to O55:H8 serotype and showed a MDR-pattern as described in section 3.3. Serogroup O55 has widely been associated with infant illness and these strains usually have pathogenic properties in common with O157:H7 (Whittam et al., 1993). All the non-O157 STEC strains harbouring *stx2* gene (6/14; 42.9%) carried subtypes $stx2_a$ or $stx2_d$ and were widely distributed in both bulk-tank milk and farm environments. Among them, strains AR10C2 and AR6C2 are associated with these clinical relevant serotypes (O91:H21 and O55:H8, respectively) and were isolated from air samples which would facilitate contamination of milk in farms.

3.3. Antimicrobial sensibility

A high number of the studied strains (14/33; 42.4%) exhibited antimicrobial resistance as shown in Table 3.

A moderate rate of aEPEC (5/18; 27.8%) and the *E. albertii* strain exhibited resistance to at least one antimicrobial substance. More than a half of the antimicrobial-resistant aEPEC shared a MDR-pattern which included aminoglycosides, tetracyclines, cephalosporins and sulfonamides. A

similar resistance pattern was also found in EPEC strains from children with acute diarrhoea (Scaletsky, Souza, Aranda, & Okeke, 2010). Two multidrug-resistant isolates (H10C1 and H4C12) were resistant to nine and eight antibiotics respectively, and harboured the bla_{TEM} gene linked to ESBL production but failed the phenotypic confirmatory test.

Eight STEC strains (57.1%) were resistant to at least one of the 22 tested antimicrobial substances. Among them, we observed MDR on three (37.5%), of which one isolate from a handler sample (M2C18) harboured the bla_{TEM} gene linked to ESBL production. Strains AR6C2 (O55:H8) and P10C6 (ONT:H1), recovered from air and feed respectively, also showed a MDR-pattern containing penicillins, cephalosporins and amynoglicosides. The MDR levels were also similarly high in indicator *E. coli* isolates from calves in reporting countries in EU (EFSA & ECDC, 2017) and their predominant MDR pattern is shared with MDR-strain M2C18. This MDR occurrence could shows extensive administration of antimicrobials over many years and it may have led to the development of multiple resistances by mobile genetic elements, resulting in co-selection. Therefore, we isolated antimicrobial-resistant STEC strains from farm environments as well as bulk-tank milk to be used for human consumption or to be transformed into dairy products. Some of them showed MDR and were isolated from handlers, air and feed.

3.4. Molecular typing of strains

Table 3 shows the classification of the 33 diarrheagenic strains through MLST and *Xba*I-PFGE, along with other key features. Both molecular typing methods were independent from antibiotic-resistance profiles. Sequence analysis yielded 13 sequence types that shows a high diversity among the tested strains. Their corresponding allelic profiles are showed in supplementary file (Table S1). Despite no new alleles were detected, two STs not reported yet in Enterobase database for *E. coli* were found.

One clonal complex, identified as ST20 Cplx according to Enterobase database, included STs 20 and 17 (Figure 1). Except for strain MK16C5 (HNM), all strains included in ST20 Cplx were associated to H2 antigen. Virulence-factor profiles of strains in ST20 Cplx and also in ST 327

were a distinctive characteristic of each respective sequence type (Table S1 -Supplementary file). Moreover, these three sequence types were the most frequent among the aEPEC strains and were obtained from milk and faecal samples recovered from multiple cow farms of different villages, indicating their wide dissemination.

It must be noted that ST 442 included two strains of aEPEC (milk sample) and STEC (air sample) which were identified as O146:H21 and O91:H21, respectively. Despite serotype O146:H21 was associated with a aEPEC strain obtained from milk, this serotype is considered to be specific to STEC (Blanco et al., 2004a), commonly found in sheep or goat's milk (Álvarez-Suárez et al., 2016; Otero et al., 2017) , and linked to human illness (EFSA & ECDC, 2016).

Regarding STEC strains, ST 10 was predominant (8/14; 57.1%) and included strains obtained from different sources. All these STEC strains in ST 10 but two belonged to the predominant serotype O140:H32 and the phylogenetic group A, and were *stx1+*. According to Enterobase database for *E. coli* (http://enterobase.warwick.ac.uk/species/ecoli/search_strains), ST 10 includes a large and diverse amount of strains, some of which are highly virulent by causing HUS and producing ESBL.

The two new sequences types were corresponding with *E. albertii* (strain H8C5) and STEC (strain AR6C2), respectively. This latter, isolated from air, with an antimicrobial-resistance profile and virulence properties, belonged to serotype O55:H8 which is recognized as human pathogen (Whittam et al., 1993).

PFGE analysis distinguished eight clusters with a minimum similarity coefficient (Dice) of 71%, named by the letters A' to H' (Figure S1 -Supplementary file). The type E' was the most heterogeneous since contained five strains of aEPEC, three strains of STEC, and the *E. albertii* strain, obtained from different origins (milk, faeces and feed samples). This analysis showed a high genetic diversity also confirmed when studying polymorphisms through MLST, as previously reported on STEC and aEPEC elsewhere (Afset et al., 2008; Otero et al., 2013). Despite STEC strains were genetically diverse, there is a relationship between the strains isolated from milk and

the isolates obtained from farm environments, with the predominant ST 10 including strains from milk, handlers and cow faeces which were not isolated from a unique cow farm (Table 3). In contrast, most of the studied aEPEC strains, associated with the predominant ST 20 (PFGE-type C') and ST 327 (PFGE-types E' and F'), were obtained from cow's milk.

4. Conclusions

This study provides further evidence that cow's milk and dairy cattle farm environments are potential sources of aEPEC and non-O157 STEC, some of which are associated with serotypes clinically significant, bearing virulence genes and multiple antibiotic resistance, that may raise public health concern due to the potential human infection and antimicrobial resistance dissemination throughout food system.

No detection of EPEC and STEC in matured cheese obtained from raw cow's milk confirms that cheesemaking process and ripening play an important role on their control.

Moreover, this is the first isolation of *E. albertii*, emerging pathogen causing human disease, from cow's faeces.

ACKNOWLEDGEMENTS

This work was supported by: "Consejería de Educación" (Junta de Castilla y Léon) and "Fondo Europeo de Desarrollo Regional" (FEDER; European Commission) [ref. LE113P17; Spain]; "Ministerio de Economía y Competitividad" and FEDER [ref. AGL2013-47852; Spain]; "Agencia Estatal de Investigación" and FEDER [ref. AGL2016-79343-R; AEI, Spain]; "Plan Estatal de I+D+I 2013-2016", "Instituto de Salud Carlos III" (ISCIII), "Subdirección General de Evaluación y Fomento de la Investigación", and FEDER [ref. PI16/01477; Spain]; "Consellería de Cultura, Educación e Ordenación Universitaria" (Xunta de Galicia, Spain) and FEDER [refs. CN2012/303 and ED431C 2017/57].

E. A. Rios, I. García-Meniño and S. Camille-Flament were supported by Cnpq – "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (Brazil), by "Conselleria de Cultura,

Educación e Ordenación Universitaria" [grant number ED481A-2015/149; Xunta de Galicia, Spain], and "Ministerio de Educación, Cultura y Deporte" [grant number FPU15/02644; Spain], respectively.

REFERENCES

Afset, J. E., Anderssen, E., Bruant, G., Harel, J., Wieler, L., & Bergh, K. (2008). Phylogenetic backgrounds and virulence profiles of atypical enteropathogenic *Escherichia coli* strains from a case-control study using multilocus sequence typing and DNA microarray analysis. *Journal of Clinical Microbiology*, *46*(7), 2280–2290. https://doi.org/10.1128/JCM.01752-07

Aidar-Ugrinovich, L., Blanco, J., Blanco, M., Blanco, J. E., Leomil, L., Dahbi, G., … Pestana de Castro, A. F. (2007). Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in São Paulo, Brazil. *International Journal of Food Microbiology*, *115*(3), 297–306. https://doi.org/10.1016/j.ijfoodmicro.2006.10.046

Altalhi, A. D., & Hassan, S. A. (2009). Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers. *Food Control*, *20*(10), 913–917. https://doi.org/10.1016/J.FOODCONT.2009.01.005

Álvarez-Suárez, M.-E., Otero, A., García-López, M.-L., Dahbi, G., Blanco, M., Mora, A., … Santos, J. A. (2016). Genetic characterization of Shiga toxin-producing *Escherichia coli* (STEC) and atypical enteropathogenic *Escherichia coli* (EPEC) isolates from goat's milk and goat farm environment. *International Journal of Food Microbiology*, *236*, 148–154. https://doi.org/10.1016/J.IJFOODMICRO.2016.07.035

Barkalita, L., Sharma, R. K., Borah, P., Kalita, D., Hussain, I., Doley, M. K., … Neher, S. (2016). Isolation and molecular characterization of shigatoxigenic and enteropathogenic *Escherichia coli* from diverse sources. *Journal of Animal Research*, *1*, 27–31. https://doi.org/10.5958/2277-940X.2016.00005.X

Blanco, J. E., Blanco, M., Alonso, M. P., Mora, A., Dahbi, G., Coira, M. A., & Blanco, J. (2004). Serotypes , virulence genes , and intimin types of shiga toxin (verotoxin)-producing Escherichia coli isolates from human patients: prevalence in Lugo, Spain , from 1992 through 1999. *Journal of Clinical Microbiology*, *42*(1), 311–319. https://doi.org/10.1128/JCM.42.1.311

Blanco, M., Blanco, J. E., Dahbi, G., Alonso, M. P., Mora, A., Coira, M. A., … Blanco, J. (2006). Identification of two new intimin types in atypical enteropathogenic *Escherichia coli*. *International Microbiology : The Official Journal of the Spanish Society for Microbiology*, *9*(2), 103–110. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16835840

Blanco, M., Blanco, J. E., Mora, A., Dahbi, G., Alonso, M. P., González, E. A., … Blanco, J. (2004). Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-xi). *Journal of Clinical Microbiology*, *42*(2), 645–651. https://doi.org/10.1128/JCM.42.2.645-651.2004

Canizalez-Roman, A., Gonzalez-Nuñez, E., Vidal, J. E., Flores-Villaseñor, H., & León-Sicairos, N. (2013). Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. *International Journal of Food Microbiology*, *164*(1), 36–45. https://doi.org/10.1016/J.IJFOODMICRO.2013.03.020

China, B., Goffaux, F., Pirson, V., & Mainil, J. (1999). Comparison of *eae*, *tir*, *espA* and *espB* genes of bovine and human attaching and effacing *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Microbiology Letters*, *178*(1), 177–182. https://doi.org/10.1111/j.1574-6968.1999.tb13775.x

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environmental Microbiology Reports*, *5*(1), 58–65. https://doi.org/10.1111/1758-2229.12019

CLSI. (2016). *Performance standards for antimicrobial disk susceptibility testing. CLSI supplement M100S*. *Clinical and Laboratory Standards Institute (CLSI)* (26th ed.). Wayne, PA.

De Buyser, M.-L., Dufour, B., Maire, M., & Lafarge, V. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *International Journal of Food Microbiology*, *67*(1–2), 1–17. https://doi.org/10.1016/S0168-1605(01)00443-3

Denamur, E., Clermont, O., & Gordon, D. (2015). Guide to the various phylogenetic classification schemes for *Escherichia coli* and the correspondence among schemes. *Microbiology*, *161*(5), 980–988. https://doi.org/10.1099/mic.0.000063

EFSA, & ECDC. (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food□borne outbreaks in 2015. *EFSA Journal*, *14*(12). https://doi.org/10.2903/j.efsa.2016.4634

EFSA, & ECDC. (2017). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA Journal*, *15*(2). https://doi.org/10.2903/j.efsa.2017.4694

EFSA Panel on Biological Hazards, B. (2011). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. *EFSA Journal*, *9*(8), 2322. https://doi.org/10.2903/j.efsa.2011.2322

EUCAST. (2013). Guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0. Retrieved December 3, 2018, from http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf

EUCAST. (2015). Antimicrobial susceptibility testing EUCAST disk diffusion method. *The European Committee on Antimicrobial Susceptibility Testing*, *5*, 1–21.

Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., … Cerf, O. (2013). Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. *International Journal of Food Microbiology*, *162*(2), 190–212. https://doi.org/http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.008

Gonzalez, A. G. ., Rosa, A. C. ., Andrade, J. R. ., & Tibana, A. (2000). Enteropathogenicity markers in *Escherichia coli* strains isolated from soft white cheese and poultry in Rio de Janeiro, Brazil. *Food Microbiology*, *17*(3), 321–328. https://doi.org/10.1006/fmic.1999.0320

Guinée, P. A. M., Jansen, W. H., Wadström, T., & Sellwood, R. (1981). *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In P. W. de Leeuw & P. A. M. Guinée (Eds.), *Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea: Proceedings of a Workshop on Diagnostic Techniques for Enteropathogenic Agents Associated with Neonatal Diarrhoea in Calves and Pigs*. Held at the Central Veterinary Institute, Department of Virology. Springer Netherlands, Dordrecht.

Gunzburg, S. T., Tornieporth, N. G., & Riley, L. W. (1995). Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *Journal of Clinical Microbiology*, *33*(5), 1375–1377. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7615759

Hernandes, R. T., Elias, W. P., Vieira, M. A. M., & Gomes, T. A. T. (2009, August 1). An overview of atypical enteropathogenic *Escherichia coli*. *FEMS Microbiology Letters*. Oxford University Press. https://doi.org/10.1111/j.1574-6968.2009.01664.x

Huys, G., Cnockaert, M., Janda, J. M., & Swings, J. (2003). *Escherichia albertii* sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *53*(3), 807–810. https://doi.org/10.1099/ijs.0.02475-0

Johnson, W. M., & Tyler, S. D. (1993). PCR detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin-1 in Staphylococcus aureus. In D. H. Persing, T. F. Smith, F. C. Tenover, & T. J. White (Eds.), *Diagnostic Molecular Microbiology. Principles and Applications* (pp. 294–299). Washington, D.C.: American Society for Microbiology.

Lindsey, R. L., Garcia-Toledo, L., Fasulo, D., Gladney, L. M., & Strockbine, N. (2017). Multiplex polymerase chain reaction for identification of *Escherichia coli*, *Escherichia albertii* and *Escherichia fergusonii*. *Journal of Microbiological Methods*, *140*, 1–4. https://doi.org/10.1016/J.MIMET.2017.06.005

Monstein, H. J., Östholm-Balkhed, Å., Nilsson, M. V., Nilsson, M., Dornbusch, K., & Nilsson, L. E. (2007). Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. *Apmis*, *115*(12), 1400–1408. https://doi.org/10.1111/j.1600-0463.2007.00722.x

Mora, A., Herrera, A., López, C., Dahbi, G., Mamani, R., Pita, J. M., … Blanco, M. (2011). Characteristics of the Shiga-toxin-producing enteroaggregative *Escherichia coli* O104:H4 German outbreak strain and of STEC strains isolated in Spain. *International Microbiology*, *14*, 121–141. https://doi.org/10.2436/20.1501.01.142

Olsen, J. ., Aabo, S., Hill, W., Notermans, S., Wernars, K., Granum, P. ., … Olsvik, Ø. (1995). Probes and polymerase chain reaction for detection of food-borne bacterial pathogens. *International Journal of Food Microbiology*, *28*(1), 1–78. https://doi.org/10.1016/0168-1605(94)00159-4

Ombarak, R. A., Hinenoya, A., Awasthi, S. P., Iguchi, A., Shima, A., Elbagory, A.-R. M., & Yamasaki, S. (2016). Prevalence and pathogenic potential of Escherichia coli isolates from raw milk and raw milk cheese in Egypt. *International Journal of Food Microbiology*, *221*, 69–76. https://doi.org/10.1016/j.ijfoodmicro.2016.01.009

Orden, J. A., Cid, D., Ruiz-Santa-Quiteria, J. A., García, S., Martínez, S., & de la Fuente, R. (2002). Verotoxin-producing *Escherichia coli* (VTEC), enteropathogenic *E. coli* (EPEC) and necrotoxigenic *E. coli* (NTEC) isolated from healthy cattle in Spain. *Journal of Applied Microbiology*, *93*(1), 29–35. https://doi.org/10.1046/j.1365-2672.2002.01649.x

Otero, V., Rodríguez-Calleja, J. M., Otero, A., García-López, M.-L., & Santos, J. A. (2013). Genetic characterization of atypical enteropathogenic *Escherichia coli* isolates from ewes' milk, sheep farm environments, and humans by

multilocus sequence typing and pulsed-field gel electrophoresis. *Applied and Environmental Microbiology*, *79*(19), 5864–5869. https://doi.org/10.1128/AEM.01809-13

Otero, V., Sánchez, S., Herrera-León, S., Rodríguez-Calleja, J.-M., Otero, A., García-López, M.-L., & Santos, J. A. (2017). Detection and characterization of Shiga toxin-producing Escherichia coli (STEC) in bulk tank ewes' milk and sheep farm environment. *Small Ruminant Research*, *154*. https://doi.org/10.1016/j.smallrumres.2017.08.002

Pagani, L., Dell'Amico, E., Migliavacca, R., D'Andrea, M. M., Giacobone, E., Amicosante, G., … Rossolini, G. M. (2003). Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of Enterobacteriaceae from a hospital in Northern Italy. *Journal of Clinical Microbiology*, *41*(9), 4264–4269. https://doi.org/10.1128/JCM.41.9.4264-4269.2003

Paton, A. W., & Paton, J. C. (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157. *J.Clin.Microbiol.*, *36*(2), 598–602.

Paton, A. W., & Paton, J. C. (2002). Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for stx1, stx2, eae, ehxA, and saa. *Journal of Clinical Microbiology*, *40*(1), 271–274. https://doi.org/10.1128/JCM.40.1.271-274.2002

Paton, A. W., Srimanote, P., Talbot, U. M., Wang, H., & Paton, J. C. (2004). A new family of potent AB(5) cytotoxins produced by Shiga toxigenic Escherichia coli. *The Journal of Experimental Medicine*, *200*(1), 35–46. https://doi.org/10.1084/jem.20040392

Pollard, D. R., Johnson, W. M., Lior, H., Tyler, S. D., & Rozee, K. R. (1990). Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. *Journal of Clinical Microbiology*, *28*(3), 540–545. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2182671

Pradel, N., Livrelli, V., De Champs, C., Palcoux, J. B., Reynaud, A., Scheutz, F., … Forestier, C. (2000). Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology*, *38*(3), 1023–1031. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10698990

Scaletsky, I. C., Souza, T. B., Aranda, K. R., & Okeke, I. N. (2010). Genetic elements associated with antimicrobial resistance in enteropathogenic *Escherichia coli* (EPEC) from Brazil. *BMC Microbiology*, *10*(1), 25. https://doi.org/10.1186/1471-2180-10-25

Scheutz, F., Teel, L. D., Beutin, L., Piérard, D., Buvens, G., Karch, H., … O'Brien, A. D. (2012). Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *Journal of Clinical Microbiology*, *50*(9), 2951–2963. https://doi.org/10.1128/JCM.00860-12

Smith, J. L., Fratamico, P. M., & Gunther, N. W. (2014). Shiga Toxin-Producing *Escherichia coli*. *Advances in Applied Microbiology*, *86*, 145–197. https://doi.org/10.1016/B978-0-12-800262-9.00003-2

Tozzoli, R., Caprioli, A., Cappannella, S., Michelacci, V., Marziano, M. L., & Morabito, S. (2010). Production of the subtilase AB5 cytotoxin by Shiga toxin-negative *Escherichia coli*. *Journal of Clinical Microbiology*, *48*(1), 178–183. https://doi.org/10.1128/JCM.01648-09

Valilis, E., Ramsey, A., Sidiq, S., & DuPont, H. L. (2018). Non-O157 Shiga toxin-producing *Escherichia coli*—A poorly appreciated enteric pathogen: Systematic review. *International Journal of Infectious Diseases*, *76*, 82–87. https://doi.org/10.1016/J.IJID.2018.09.002

Verraes, C., Vlaemynck, G., Van Weyenberg, S., De Zutter, L., Daube, G., Sindic, M., … Herman, L. (2015). A review of the microbiological hazards of dairy products made from raw milk. *International Dairy Journal*, *50*, 32–44. https://doi.org/10.1016/J.IDAIRYJ.2015.05.011

Wang, G., Clark, C. G., & Rodgers, F. G. (2002). Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. *Journal of Clinical Microbiology*, *40*(10), 3613–3619. https://doi.org/10.1128/JCM.40.10.3613-3619.2002

Whittam, T. S., Wolfe, M. L., Wachsmuth, I. K., Orskov, F., Orskov, I., & Wilson, R. A. (1993). Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infection and Immunity*, *61*(5), 1619–1629. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7682992

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., … Achtman, M. (2006). Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Molecular Microbiology*, *60*(5), 1136–1151. https://doi.org/10.1111/j.1365-2958.2006.05172.x

Table 1. Distribution of positives samples for STEC and/or EPEC[(a)] in accordance to their origin (cows' milk, cheese and farm environment) and season.

Season	Origin	Tested samples (n)	STEC		EPEC[(a)]	
			Positive samples	Confirmed isolates	Positive samples	Confirmed isolates
Winter	Cows' milk	107	2	2	4	4
	Cheese	108	0	0	0	0
	Faeces	5	1	2	2	2
	Feed	5	0	0	1	1
	Air	5	0	0	0	0
	Handlers	5	2	3	0	0
	Water	5	0	0	0	0
	Milk filter	3	0	0	0	0
	subtotal	*243*	*5*	*7*	*7*	*7*
Summer	Cows' milk	107	3	3	9	9
	Cheese	108	0	0	0	0
	Faeces	10	1	1	3	3[(a)]
	Feed	10	1	1	0	0
	Air	5	2	2	0	0
	Handlers	5	0	0	0	0
	Water	10	0	0	0	0
	Milk filter	4	0	0	0	0
	subtotal	*259*	*7*	*7*	*12*	*12*[(a)]
Total		*502*	*12*	*14*	*19*	*19*[(a)]

[(a)] Positives samples for EPEC include one positive sample with a confirmed isolate which was finally identified as *Escherichia albertii*.

Table 2. Serotypes and genetic characteristics of 33 isolates (aEPEC, *E. albertii* and non-O157 STEC) from different sources in dairy cattle farms.

	Serotype	Number of isolates	Genetic profile	Phylogenetic group	Source
aEPEC	O156:H8	5	$eae_{\gamma 2}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}$	B1	Cows' milk
	O25:H2	3	$eae_{\beta 1}/espA_{\beta}/espB_{\beta}/tir_{\beta}$	B1	Cows' milk
	O15:H2	2	$eae_{\beta 1}$ /$espA_{\beta}/espB_{\beta}/tir_{\beta}$	B1	Cows' milk
	O4:H2	2	$eae_{\varepsilon 1}/espA_{\beta}/espB_{\beta}/tir_{\beta}/ehlyA$	B1	Faeces
	O25:H8	1	$eae_{\gamma 2}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}$	B1	Faeces
	O51:HNM	1	$eae_{\beta 1}/espA_{\beta}/espB_{\beta}/tir_{\beta}$	B1	Cows' milk
	O96:H7	1	$eae_{\gamma 2}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}$	B2	Faeces
	O109:H25	1	$eae_{\zeta 1}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}/ehlyA$	B1	Cows' milk
	O109:HNM	1	$eae_{\gamma 2}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}$	B1	Feed
	O146:H21	1	$eae_{\gamma 2}/espA_{\alpha}/espB_{\alpha}/tir_{\alpha}$	B1	Cows' milk
E. albertii	O69:HNM	1	$eae_{\gamma 2}/espA_{\beta}/espB_{\beta}/tir_{\beta}$	-	Faeces
STEC	O140:H32	6	$stx1_{c}$ *or* $stx1_{a}$	A	Cows' milk/Faeces/Handlers
	O2:HNM	1	$stx2_{\alpha}/ehlyA$	A	Cows' milk
	O3:HNM	1	$stx1_{c}/tia$	A	Cows' milk
	O55:H8	1	$stx2_{d}/ehlyA/tia$	B1	Air
	O91:H21	1	$stx1_{\alpha}/stx2_{\alpha}/stx2_{d}/ehlyA$	B1	Air
	O130:H21	1	$stx1_{\alpha}/stx2_{d}/ehlyA/SubAB$	B1	Feed
	O136:H1	1	$stx2_{\alpha}/ehlyA$	A	Faeces
	O156:H4	1	$stx2_{d}$	A	Handlers
	ONT:HNM	1	$stx1_{c}/ehlyA$	A	Cows' milk

Table 3. Comparison of genotypic characteristics, antimicrobial susceptibility and origin of 33 strains (aEPEC, *E. albertii* and non-O157 STEC) isolated from cows' milk, cheese and farm environment in Northwest Spain. A clonal complex grouping ST17 and ST20 is marked in discontinuous-line square.

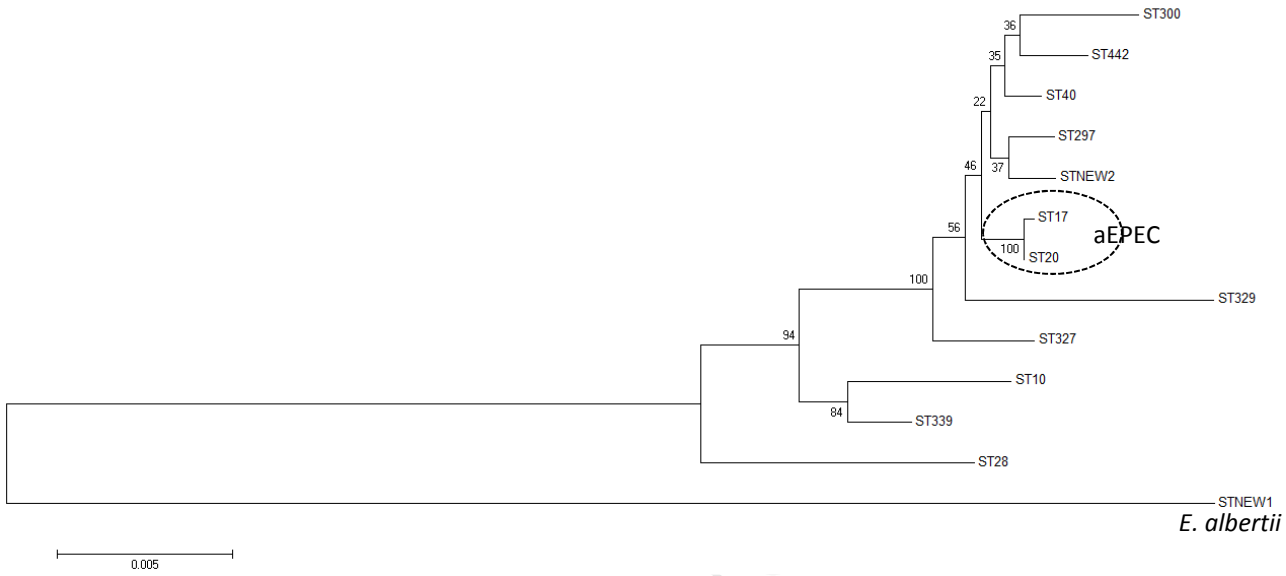
	Strain	ST[(a)]	PFGE[(b)]	Serotype	Ph. Gr.[(c)]	Resistance pattern[(d)]	Farm[(e)]	Source
aEPEC	H10C1	17	C'	O4:H2	B1	[(f)] AMP/S/KF/TE/AMC/SXT/SSS/TIC/PRL	F-B	Faeces
	H4C12	17	C'	O4:H2	B1	[(f)] AMP/S/TE/AMC/SXT/SSS/TIC/PRL	F-B	Faeces
	MK50C8	20	C'	O15:H2	B1	-	F-C	Milk
	MK7C17	20	C'	O15:H2	B1	-	F-F	Milk
	MK127C9	20	C'	O25:H2	B1	-	F-B	Milk
	MK212C3	20	C'	O25:H2	B1	-	F-O	Milk
	MK130C20	20	C'	O25:H2	B1	-	F-J	Milk
	MK16C5	20	C'	O51:HNM	B1	-	F-H	Milk
	Ha8C4	28	D'	O96:H7	B2	KF	F-D	Faeces
	P4C16	40	E'	O109:HNM	B1	-	F-B	Feed
	MK13C16	300	E'	O109:H25	B1	-	F-G	Milk
	MK110C3	327	F'	O156:H8	B1	-	F-I	Milk
	MK150C20	327	F'	O156:H8	B1	-	F-K	Milk
	MK169C17	327	F'	O156:H8	B1	CN	F-M	Milk
	MK163C15	327	F'	O156:H8	B1	S/TE/SXT/SSS	F-L	Milk
	MK116C9	327	E'	O156:H8	B1	-	F-C	Milk
	H5C24	327	E'	O25:H8	B1	-	F-E	Faeces
	MK202C5	442	E'	O146:H21	B1	-	F-N	Milk
E. albertii	H8C5	New1	E'	O69:HNM	-	AMP/KF/AMC	F-D	Faeces
STEC	MK116C19	10	A'	O140:H32	A	-	F-C	Milk
	MK37C14	10	E'	O140:H32	A	S/CN/C/	F-A	Milk
	H5C12	10	G'	O140:H32	A	-	F-E	Faeces
	M5C1	10	G'	O140:H32	A	-	F-E	Handler
	M5C4	10	G'	O140:H32	A	CN/K	F-E	Handler
	H5C2	10	G'	O140:H32	A	KF	F-E	Faeces
	MK136C13	10	A'	O2:HNM	A	-	F-E	Milk
	M2C18	10	A'	O156:H4	A	[(f)]AMP/S/KF/NA/TE/SXT/C/CN/CIP/SSS/TIC/PRL	F-A	Handler
	P10C6	297	E'	O130:H21	B1	AMP/KF/CN	F-B	Feed
	MK126C1	329	E'	O3:HNM	A	-	F-D	Milk
	Ha10C3	329	A'	O136:H1	A	KF/AMC	F-B	Faeces
	MK40C20	339	H'	ONT:HNM	A	-	F-B	Milk
	AR10C2	442	G'	O91:H21	B1	AMP/KF/AMC	F-B	Air
	AR6C2	New2	B'	O55:H8	B1	AMP/KF/AMC/CN	F-C	Air

[(a)]Sequence type through MLST; [(b)]*Xba*I-PFGE type; [(c)]Ph.Gr., Phylogenetic Group; [(d)]Tested antimicrobials: AMC, amoxicillin-clavulanic acid; AMP, ampicillin; ATM, aztreonam; C, chloramphenicol; CAZ, ceftazidime; CEC, cefaclor; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; CXM, cefuroxime; IPM, imipenem; K, kanamycin; KF, cephalothin; NA, nalidixic acid; PRL, piperacillin; S, streptomycin; SSS, compound sulphonamides; SXT, sulfamethoxazole/trimethoprim; TE, tetracyclines; TIC, ticarcillin; FOX, cefoxitin; FEP, cefepime; [(e)]Dairy cattle farm identification; [(f)]Strain bla_{TEM}+.

Figure captions

Figure 1. Neighbor-joining tree based on the concatenated nucleotide sequences of the seven *loci* in 33 strains of diarrheagenic *E. coli* and *E. albertii*. Bootstrapping values are shown in branch nodes and a clonal complex is marked by discontinuous-line circle.

Figure 1.



In cows' milk, occurrence is high (>6%) for aEPEC whereas ca. 2% for STEC

No detection of diarrheagenic *E. coli* in cheese obtained from raw cows' milk

Spanish cows' milk is source of high-diverse aEPEC with multiple antibiotic resistance

Milk and farm environment are sources of non-O157 STEC with clinical importance

Isolation of the emerging human enteropathogen *E. albertii* in a dairy cattle farm