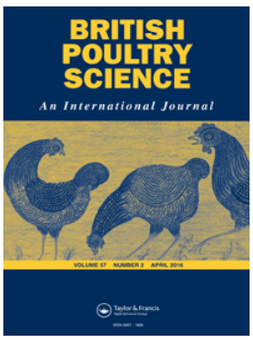

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Isolation of atypical enteropathogenic *Escherichia coli* from chicken and chicken-derived products

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Abstract 1. Atypical enteropathogenic *Escherichia coli* (EPEC) strains from chicken and chicken-derived products were isolated and characterised.
2. The strains presented a wide variety of serotypes, some have been reported in other animal species (O2:H40, O5:H40) and in children with diarrhoea (O8:H-). Most of the strains carried intimin β.
3. The results indicate that chicken and chicken products are important sources of atypical EPEC strains that could be associated with human disease, and highlight the need to improve hygiene practices in chicken slaughtering and meat handling.

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) causes diarrhoea in children of less than 2 years of age and is responsible for outbreaks and cases of prolonged diarrhoea worldwide (Trabulsi *et al.*, 2002; Nguyen *et al.*, 2006).

EPEC has the ability to cause attaching and effacing lesions produced by intimin (encoded by the *eae* gene) and other proteins. Some *E. coli* strains that produce Shiga toxins (encoded by *stx*1 and *stx*2 genes) can also produce attaching and effacing lesions. Therefore, EPEC strains are those that are *eae* positive but negative for *stx*.

The C-terminal end of intimin is the variable region responsible for receptor binding and it has been suggested that different intimin types are responsible for different host tissue cell tropism. Many intimin subtypes have been described, such as α_1, α_2, β_1, β_2, γ_1, γ_2/θ, δ/κ, ε, ζ, η, η2, *t*, λ, μ, ν, ξ and o (Oswald *et al.*, 2000; Blanco *et al.*, 2004).

Some EPEC strains can harbour a type IV pilus (bundle-forming pilus, BFP) with a major structural subunit encoded by the *bfpA* gene, present in the EPEC adherence plasmid. According to the presence or the absence of this pilus, EPEC strains are classified as typical and atypical, respectively (Trabulsi *et al.*, 2002). The most common serotypes of typical EPEC are O55:H6, O55:H-, O86:H34, O111:H2, O111:H-, O119:H6, O127:H6, O127:H40, O142:H6 and O142:H34, while O26:H11, O55:H7, O55:H34, O86:H8, O111ac:H9, O111:H25, O119:H2, O125ac:H6 and O128ac:H2 are the most frequent serotypes of atypical EPEC (Trabulsi *et al.*, 2002; Hernandes *et al.*, 2009). In addition, Moreira *et al.* (2008) showed that serotype O51:H40 comprises mostly atypical EPEC strains, but also some typical EPEC strains.

EPEC transmission occurs by the faecal–oral route, through contaminated hands, water and food. Considering food, EPEC has been found in pasteurised milk, soft cheese and different meats (Carneiro *et al.*, 2006; Najand and Ghanbarpour, 2006; Lee *et al.*, 2009), but there is scarce information about the presence in chicken and chicken products. Therefore, the aim of this study was to isolate and characterise EPEC strains from chicken and chicken-derived products.

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MATERIAL AND METHODS

EPEC isolation

A total of 237 samples collected from cloacae of living chickens, carcasses, giblets and hamburgers previously identified as positive for *eae* by PCR (Alonso *et al.*, 2012) were cultured on MacConkey agar plates by incubating at 37°C for 24 h. Then 10 to 200 separate colonies/sample were analysed by a multiplex PCR to detect *stx*$_1$, *stx*$_2$, *eae*, *saa* and *ehxA* genes using the PCR protocol and primers described by Paton and Paton (2002). In addition, isolates were confirmed as *E. coli* by PCR amplification of the universal stress protein (*uspA*) (Chen and Griffiths, 1998). Amplification products were electrophoresed in 2% agarose gels and stained with ethidium bromide.

As several samples were contaminated with *Proteus*, subsequent cultures were performed in CLED agar (Cysteine lactose electrolyte deficient) to obtain pure colonies of *E. coli*. Afterwards, the absence of *Proteus* was verified by culture on agar plates with a non-selective medium (Trypticase Soya Agar).

Each EPEC isolate (*eae* positive and *stx* negative) was tested for the presence of *bfpA* gene by monoplex PCR using the method of Gunzburg *et al.* (1995).

Serotyping

Determination of the O-antigens was performed by the microagglutination technique described by Guinée *et al.* (1981) and modified by Blanco *et al.* (1996) using a kit of 70 antisera provided by the Laboratorio de Referencia de *E. coli* (LREC) (Lugo, Spain). H antigens were determined by tube agglutination technique with 56 antisera (Statens Serum Institute, Copenhagen, Denmark) (Orskov and Orskov, 1984) and the non-typeable strains (ON:HNT) were tested at the Instituto Adolfo Lutz (Sao Paulo, Brazil) by a tube agglutination test (Ewing, 1986) using O (O1–O181) and H (H1–H56) antisera.

Intimin subtyping

The subtyping method described by Oswald *et al.* (2000) was used for the identification of *eae* gene variants (α, β, γ and ε). This method is based on several PCR reactions with the same "forward primer" and different "reverse primers", which are specific for each variant.

RESULTS

A total of 45 EPEC isolates were obtained. These were isolated from cloacae (15 isolates from 13 EPEC positive samples), carcasses (9 isolates from 6 EPEC positive samples), giblets (12 isolates from the same number of EPEC positive samples) and chicken hamburgers (9 isolates from the same number of EPEC positive samples).

EPEC isolates were negative for *bfpA* and *saa* genes and only one harboured *ehxA* gene (Table).

A wide variety of O antigens were obtained from chicken and chicken-derived products: O2, O3, O5, O8, O19, O40, O103, O109, O119, O123, O130, O136, O159, O166, O177 and OR combined with 15 different H antigens (H4, H6, H9, H10, H11, H19, H26, H28, H32, H40, H45, H51). Ten

Table. *Serotypes and eae variants of atypical enteropathogenic* Escherichia coli *isolated from cloacae, carcasses and giblets of chickens and chicken hamburgers.*

Origin	Serotype[1]	*eae* variant
Cloacae	O8:H-	β
	O40:H10	β
	O40:H10	β
	O40:H10[a]	β
	O40:H10	β
	O40:H10	β
	O103:H19	ND
	O103:H-	ND
	O119:H4	β
	O130:H11[b]	ND
	O159:H28	ND
	ONT:H10	β
	ONT:H40[b]	γ
	ONT:HNT[a]	β
	O?:H6	ND
Carcasses	O2:H40	β
	O3:H26[c]	β
	O5:H40[d]	β
	O5:H-[d]	β
	O19:H11[e]	β
	O123:H32	ND
	ONT:H10	β
	ONT:H11[e]	β
	ONT:H26[c]	β
Giblets	O2:H-	ND
	O3:H40	β
	O40:H10	β
	O40:H10	β
	O40:H-	β
	O40:H-	β
	O136:H26	β
	O166:H45	β
	OR:H32	β
	ONT:H9	ND
	ONT:H51	β
	ONT:H-	β
Hamburgers	O40:H10	β
	O40:H10	β
	O40:H10	β
	O40:H10	β
	O40:H-	β
	O109:H32	ND
	O136:H26	β
	O177:H11*	β
	ONT:H51	β

[1]Isolates with the same superscript letter belong to the same sample.
ND: not determined (negative for α, β, γ and ε).
*This isolate was also positive for the *ehxA* gene.

isolates were considered ONT (O non-typeable), one was classified as O? (it reacted with more than one antisera with the same titer), 8 non-motile and 1 HNT (H non-typeable) (Table).

Different serotypes were detected in two samples from cloacae (O40:H10 and ONT:HNT in one sample, and O130:H11 and ONT:H40 in another) and three samples from carcasses (O3:H26 and ONT:H26, O5:H40 and O5:H-, O19:H11 and ONT:H11) (Table).

Most of the EPEC isolates carried intimin β irrespective of their origin (cloacae, carcasses, giblets or hamburgers). This variant was observed in more than 15 serotypes. Only one isolate from cloacae harboured intimin γ. Nine isolates, corresponding to diverse serotypes and origins, were negative for intimin α, β, γ and ε (Table).

DISCUSSION

No typical EPEC strains were detected in the chicken samples analysed in agreement with Kobayashi *et al.* (2002), Krause *et al.* (2005) and Farooq *et al.* (2009). This finding is also consistent with Trabulsi *et al.* (2002) who reported the isolation of atypical EPEC serotypes mostly from animal hosts although it has been isolated from children with diarrhoea and gastroenteritis (Hernandes *et al.*, 2009).

Most of EPEC isolates carried intimin β, in agreement with Kobayashi *et al.* (2002) who detected this variant in atypical EPEC strains from broiler chicken cloacae. This variant is widely distributed in EPEC isolates from human and several animal species such as cattle, pigs, rabbits, dogs and birds (Oswald *et al.*, 2000). Only one isolate harboured intimin γ and the remaining isolates were negative for α, β, γ and ε variants. These data suggest that these atypical EPEC isolates may carry intimin variants that are found less frequently or correspond to new ones.

In the present study several new serotypes were detected for EPEC isolates. These novel serotypes are: O2:H-, O3:H26, O3:H40, O19:H11, O40:H10, O40:H-, O103:H19, O109:H32, O123:H32, O130:H11, O136:H26, O159:H28, O166:H45, OR:H32. Some of these serotypes (O40:H10, O40:H- and O136:H26) were detected more than once, O40:H10 being the most prevalent.

Certain EPEC serotypes isolated from chicken carcasses have also been reported in sheep (O2:H40), goats (O5:H40) and pigs (O2:H40) (Cortés *et al.*, 2005; Fröhlicher *et al.*, 2008). Furthermore, EPEC belonging to serotype O8:H- has also been isolated from children with diarrhoea in Germany, indicating its pathogenic potential (Bielaszewska *et al.*, 2008). Furthermore, serotype O119:H4 has also been reported in EPEC from children with diarrhoea but it is not possible to determine if it corresponded to atypical EPEC as the authors did not look for the *bfpA* gene (Aslani and Alikhani, 2009).

Interestingly, some EPEC isolates from the present study belong to serotypes O5:H-, O103:H- and O177:H11 which have been reported as *eae*-positive shigatoxigenic *E. coli* (STEC) in human, cattle and ground beef (Beutin *et al.*, 2004; Hussein and Bollinger, 2005; Fernández *et al.*, 2012), and have been associated with haemolytic-uraemic cases in several countries (Bettelheim, 2007). This is important because several researchers postulate that STEC as well as typical EPEC may become atypical EPEC due to the loss of mobile genetic elements such as bacteriophages or plasmids coding Stx and BFP, respectively, during infection (Bielaszewska *et al.*, 2008; Hernandes *et al.*, 2009). Moreover, one chicken hamburger isolate was positive for both *eae* and *ehxA* (plasmidic gene), consistent with Wani *et al.* (2004), who detected strains harbouring this genotype from chicken faeces.

In this study, atypical EPEC strains were obtained from chicken suggesting that it could be a reservoir of these bacteria. Their presence in chicken products is evidence that contamination can occur during slaughtering and manufacturing processes, representing a risk for humans as some serotypes detected in this study have been associated with diarrhoea. These results highlight the need to improve hygiene practices in chicken slaughtering and meat handling to avoid the presence of EPEC at retail stores.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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REFERENCES

Alonso, M.Z., Lucchesi, P.M.A., Rodríguez, E.M., Parma, A.E. & Padola, N.L. (2012) Enteropathogenic (EPEC) and Shigatoxigenic *Escherichia coli* (STEC) in broiler chickens and derived products at different retail stores. *Food Control*, **23**: 351–355. doi:10.1016/j.foodcont.2011.07.030

Aslani, M.M. & Alikhani, M.Y. (2009) Serotypes of enteropathogenic *Escherichia coli* isolated from children under 5 years of age. *Iranian Journal of Public Health*, **38**: 70–77.

Bettelheim, K.A. (2007) The non-O157 Shiga-toxigenic (Verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Critical Reviews in Microbiology*, **33**: 67–87. doi:10.1080/10408410601172172

Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S. & Gleier, K. (2004) Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. *Journal of Clinical Microbiology*, **42**: 1099–1108. doi:10.1128/JCM.42.3.1099-1108.2004

Bielaszewska, M., Middendorf, B., Köck, R., Friedrich, A.W., Fruth, A., Karch, H., Schmidt, M.A. & Mellmann, A. (2008) Shiga toxin-negative attaching and effacing *Escherichia coli*: distinct clinical associations with bacterial phylogeny and virulence traits and inferred in-host pathogen evolution. *Clinical Infectious Diseases*, **47**: 208–217. doi:10.1086/591151}

Blanco, J.E., Blanco, M., Blanco, J., Mora, A., Balaguer, L., Mouriño, M., Juárez, A. & Jansen, W.H. (1996) O serogroups, biotypes and *eae* genes in *Escherichia coli* isolates from diarrheic and healthy rabbits. *Journal of Clinical Microbiology*, **34**: 3101–3107.

Blanco, M., Blanco, J.E., Mora, A., Dahbi, G., Alonso, M.P., González, E.A., Bernárdez, M.I. & 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-ξ). *Journal of Clinical Microbiology*, **42**: 645–651. doi:10.1128/JCM.42.2.645-651.2004

Carneiro, L.A.M., Lins, M.C., Garcia, F.R.A., Silva, A.P.S., Mauller, P.M., Alves, G.B., Rosa, A.C.P., Andrade, J.R.C., Freitas-Almeida, A.C. & Queiroz, M.L.P. (2006) Phenotypic and genotypic characterisation of *Escherichia coli* strains serogrouped as enteropathogenic *E. coli* (EPEC) isolated from pasteurised milk. *International Journal of Food Microbiology*, **108**: 15–21. doi:10.1016/j.ijfoodmicro.2005.10.010

Chen, J. & Griffiths, M.W. (1998) PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. *Letters in Applied Microbiology*, **27**: 369–371. doi:10.1046/j.1472-765X.1998.00445.x

Cortés, C., De La Fuente, R., Blanco, J., Blanco, M., Blanco, E., Dhabi, G., Mora, A., Justel, P., Contreras, A., Sánchez, A., Corrales, J.C. & Orden, J.A. (2005) Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. *Veterinary Microbiology*, **110**: 67–76. doi:10.1016/j.vetmic.2005.06.009

Ewing, W.H. (1986) *Edwards & Ewing's Identification of Enterobacteriaceae*. 4th edn. (New York, Elsevier Science Publishing Co., Inc).

Farooq, S., Hussain, I., Bhat, M.A. & Wani, S.A. (2009) Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India. *Letters in Applied Microbiology*, **48**: 692–697.

Fernández, D., Sanz, M.E., Parma, A.E. & Padola, N.L. (2012) Short communication: characterization of Shiga toxin-producing *Escherichia coli* isolated from newborn, milk-fed, and growing calves in Argentina. *Journal of Dairy Science*, **95**: 5340–5343. doi:10.3168/jds.2011-5140

Fröhlicher, E., Krause, G., Zweifel, C., Beutin, L. & Stephan, R. (2008) Characterization of attaching and effacing *Escherichia coli* (AEEC) isolated from pigs and sheep. *BMC Microbiology*, **8**: 144. doi:10.1186/1471-2180-8-144

Guinée, P.A.M., Jansen, H.W., Wasdtröm, T. & Sellwood, R. (1981) *Escherichia coli* associated with neonatal diarrhoea in piglets and calves, in: De Leew, P.W. & Guinée, P.A.M. (Eds) *Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea: Current Topics in Veterinary and Animal Science*, pp. 126–162 (The Hague, Martinus Nijhoff).

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**: 1375–1377.

Hernandes, R.T., Elias, W.P., Vieira, M.A.M. & Gomes, T.A.T. (2009) An overview of atypical enteropathogenic *Escherichia coli*. *FEMS Microbiology Letters*, **297**: 137–149. doi:10.1111/j.1574-6968.2009.01664.x

Hussein, H.S. & Bollinger, L.M. (2005) Prevalence of Shiga toxin-producing *Escherichia coli* in beef. *Meat Science*, **71**: 676–689. doi:10.1016/j.meatsci.2005.05.012

Kobayashi, H., Pohjanvirta, T. & Pelkonen, S. (2002) Prevalence and characteristics of intimin and Shiga toxin-producing *Escherichia coli* from gulls, pigeons and broilers in Finland. *Journal of Veterinary Medical Science*, **64**: 1071–1073. doi:10.1292/jvms.64.1071

Krause, G., Zimmermann, S. & Beutin, L. (2005) Investigation of domestic animals and pets as a reservoir for intimin (*eae*) gene positive *Escherichia coli* types. *Veterinary Microbiology*, **106**: 87–95. doi:10.1016/j.vetmic.2004.11.012

Lee, G.Y., Jang, H.I., Hwang, I.G. & Rhee, M.S. (2009) Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. *International Journal of Food Microbiology*, **134**: 196–200. doi:10.1016/j.ijfoodmicro.2009.06.013

Moreira, F.C., Vieira, M.A.M., Ferreira, A.J.P., Girão, D.M., Vaz, T.M.I., Rosa, A.C.P., Knobl, T., Irino, K., Freymüller, E. & Gomes, T.A.T. (2008) *Escherichia coli* strains of serotype O51:H40 comprise typical and atypical enteropathogenic *E. coli* strains and are potentially diarrheagenic. *Journal of Clinical Microbiology*, **46**: 1462–1465. doi:10.1128/JCM.01854-07

Najand, L.M. & Ghanbarpour, R. (2006) A study on enteropathogenic *Escherichia coli* isolated from domestic Iranian soft cheese. *Veterinarski Arhiv*, **76**: 531–536.

Nguyen, R.N., Taylor, L.S., Tauschek, M. & Robins-Browne, R.M. (2006) Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerging and Infectious Diseases*, **12**: 597–603. doi:10.3201/eid1204.051112

Orskov, F. & Orskov, I. (1984) Serotyping of *Escherichia coli*, in: Bergan, T. (Ed) *Methods in Microbiology*, Vol. **14**, pp. 43–112 (London, Academic Press).

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O. & Caprioli, A. (2000) Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. *Infection and Immunity*, **68**: 64–71. doi:10.1128/IAI.68.1.64-71.2000

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**: 271–274. doi:10.1128/JCM.40.1.271-274.2002

Trabulsi, L.R., Keller, R. & Tardelli Gomes, T.A. (2002) Typical and atypical enteropathogenic *Escherichia coli*. *Emerging and Infectious Diseases*, **8**: 508–513. doi:10.3201/eid0805.010385

Wani, S.A., Samanta, I., Bhat, M.A. & Nishikawa, Y. (2004) Investigation of Shiga toxin-producing *Escherichia coli* in avian species in India. *Letters in Applied Microbiology*, **39**: 389–394. doi:10.1111/lam.2004.39.issue-5