Assessment of Virulence Factors Characteristic of Human *Escherichia coli* Pathotypes and Antimicrobial Resistance in O157:H7 and Non-O157:H7 Isolates from Livestock in Spain


The distribution of virulence factors (VF) typical of diarrheagenic *Escherichia coli* and the antimicrobial resistance (AMR) profiles were assessed in 780 isolates from healthy pigs, broilers, and cattle from Spain. VF distribution was broader than expected, although at low prevalence for most genes, with AMR being linked mainly to host species.

Pathogenic isolates of *Escherichia coli* are characterized by the presence of virulence factors (VF) that can be combined, leading to different pathotypes (1). Six distinct intestinal pathotypes have been differentiated, including enteropathogenic (*EPEC*), enterotoxigenic (*ETEC*), enterohemorrhagic (*EHEC*), enteroinvasive (*EIEC*), and diffusely adherent (*DAEC*) *E. coli* (2). The main reservoirs for *EPEC*, *EAE*, and *EIEC* are humans (1, 3). In contrast, *EHEC* transmission from animals to humans may also occur through fecal contamination of food or water. In addition to the conventional *E. coli* pathotypes, novel hybrid pathotypes may emerge, as occurred with *EAE*/*EHEC* O104:H4 of the German outbreak, which spread across several European countries, affecting almost 4,000 people and causing more than 50 deaths (4).

Limited information on the distribution of *E. coli* pathotypes in healthy livestock (especially non-Shiga toxin-producing *E. coli* [STEC]) and the possible association between the carriage of VF and their antimicrobial resistance (AMR) patterns is available. In this study, we evaluated the distribution of VFs and AMR profiles in *E. coli* isolates recovered from healthy livestock in Spain.

All these *E. coli* isolates were recovered in 2009 (n = 780) through the Spanish Surveillance Network of Antimicrobial Resistance in Bacteria of Veterinary Origin (VAV Network) (5). Isolate distribution was as follows: 50 O157:H7 *E. coli* strains from cattle and 730 *E. coli* strains (278 from pigs, 196 from broilers, and 256 from cattle) belonging to other serotypes (referred to here as non-O157:H7 *E. coli*) regardless whether they were *EHEC*. These isolates were recovered from pooled samples collected at Spanish slaughterhouses selected according to their slaughter capacity and located in different regions within the country. Isolates were obtained by culturing pooled feces samples from pigs (2 animals per pool, 556 individual fecal samples analyzed), cattle (2 animals per pool, 512 individual fecal samples analyzed) and broilers (3 animals per pool, 588 individual fecal samples analyzed). Each pool represented one slaughter batch from one single farm.

Samples from pigs, broilers, and cattle were cultured on MacConkey agar plates (6). In addition, cattle feces were processed to obtain *E. coli* O157:H7 according to the ISO 16.654:2001 protocol.

Nine VFs (*stx*₁, *stx*₂, *eae*, *chxA*, heat-stable enterotoxin [ST], heat-labile enterotoxin [LT], *bfpA*, *plm*, and *aggR*) and the somatic and flagellar antigens of O157:H7 and O104:H4 serotypes were assessed in all isolates using conventional PCR (see Table S1 in the supplemental material). The β-D-glucuronidase-encoding gene *uidA* was also included for *E. coli* confirmation (7). In addition, the *stx*₂ PCR product from two swine isolates was sequenced to assess their *stx*₂ type (8). All isolates were also tested against 14 antibiotics by broth microdilution (6).

All isolates were *E. coli*, as demonstrated by the positive *uidA* results, and all were negative for *bfpA*, LT, *aggR*, and *wzx*₁₀₄/~*fliC*₄₄ (data not shown). Distribution of VFs varied depending on the host species, with pigs and cattle presenting more diversity of genotypes, while 57 (29.1%) of the isolates from broilers carried *eae* as the only detected VF, in agreement with previous reports on avian isolates (9).

In cattle *E. coli*, the occurrence of *eae* (3.9%) and Shiga toxins (7.7%) in this study was lower among our strains than in previous works (10, 11, 12).

In pigs prevalence of VF was also low, with one isolate being *plm* positive and two isolates being positive for both *stx*₂ and ST (Table 1). In fact, a significant association for the concurrent presence of *stx*₂ and ST was found in both cattle and swine isolates (Fisher’s exact test, *P* < 0.001). Interestingly, this combination had not been previously described for cattle. The sequencing of the *stx* gene from the two ST⁺ *stx*₂⁺ strains identified them as *stx*₂e. In previous studies, this pattern was detected in healthy pigs and piglets suffering from postweaning diarrhea (13, 14). Our *stx*₂e/ST-positive strains were obtained from adult healthy pigs, highlighting their possible role as asymptomatic reservoirs of this intermediate strain.

Regarding *E. coli* O157:H7 strains, 100% of the isolates were...
positive for at least three of the typical EHEC VFs (ehxA, eae, and stx2), confirming the role of cattle as a relevant reservoir of this pathotype and a public health concern, as previously described (15).

Almost 20% of the isolates showed no resistance to the studied antimicrobials, and no resistance to colistin was detected (see Table S1 in the supplemental material). The highest resistance rates were found for tetracycline, streptomycin, and sulfonamides, especially in pig isolates. However, the largest proportion of isolates resistant to quinolones and beta-lactams, including third-generation cephalosporins, was observed in avian isolates. Bovine strains showed lower AMR percentages, although cattle E. coli O157:H7 isolates accounted for the highest levels of resistance to gentamicin, kanamycin, and chloramphenicol (see Table S1). Significant differences (Pearson chi-square test, P < 0.05) in the proportion of resistant isolates to the different antimicrobials, and no resistance to colistin was detected (see Table S1).

The homogeneity of the limited VF distribution found in pigs and broilers made it impossible to detect resistance patterns related to VF combinations in these animal species. In contrast, differences in AMR between E. coli O157:H7 and non-O157:H7 E. coli strains for all the antimicrobials except florfenicol (P = 0.49) and third-generation cephalosporins (no resistant cattle isolates).

Compared with previous data (16), resistance levels among our E. coli isolates from pigs and broilers were moderate to high for some antibiotics while they were low in the case of bovine E. coli isolates. A higher proportion of resistance to gentamicin, ciprofloxacin, tetracycline and nalidixic acid was observed in the O157:H7 E. coli cattle isolates analyzed in this study compared with previous reports.

The homogeneity of the limited VF distribution found in pigs and broilers made it impossible to detect resistance patterns related to VF combinations in these animal species. In contrast, differences in AMR between E. coli O157:H7 and non-O157:H7 isolates from cattle indicate that, although both types of strains were theoretically under the same selective pressure, there may be a specific mechanism that makes O157:H7 E. coli strains more resistant than non-O157:H7 E. coli strains. However, comparison of non-O157:H7 E. coli isolates revealed a strong association with the host species.

In summary, the selected VFs, commonly regarded in the literature as specific to a given E. coli pathotype, showed a broader distribution than expected among healthy livestock in Spain, although most of the VFs analyzed were present at low frequencies. The assessment of the presence of potentially pathogenic E. coli (not only EHEC) in healthy animals could be a useful tool to evaluate and predict the risk of the emergence of new pathogenic strains from animal reservoirs.

**ACKNOWLEDGMENTS**

This work was partially supported by The Spanish Ministry of Agriculture, Food and Environment and by the Autonomous Community of Madrid, Spain (S0505/AGR-0265; S2009/AGR-1489). J. Álvarez is a recipient of a Sara Borrell postdoctoral contract (CD11/00261, Ministerio de Ciencia e Innovación).

We thank our technicians, M. Carmen Comerón, Nisrin Maasoumi, and Lorena del Moral, for their excellent work. We also thank Silvia Herrera-León from the National Center of Microbiology (Institute of Health Carlos III), Madrid, Spain, for providing an EPEC strain and Martina Bielaszewska from the University of Münster for providing ETEC, EIEC, and EAEC strains and the O104:H4 EAEC strain of the German outbreak to be used as positive controls in the molecular analyses.

This article is a contribution to EU FP7 ANTIGONE (project number 278976).

**REFERENCES**


