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Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans

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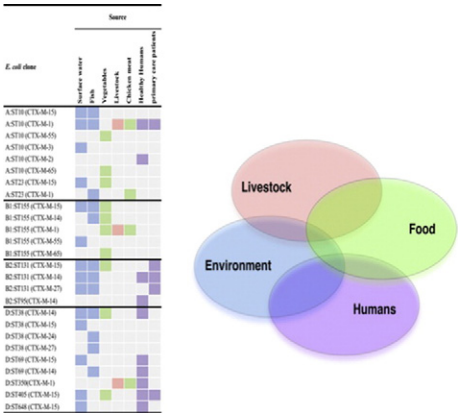
HIGHLIGHTS

- Uropathogenic ESBL-producing *Escherichia coli* were found in samples from all sources.
- Some UPEC clones are shared by these different sources: e.g., clone A:ST10 (CTX-M-1).
- Identification of clonal overlaps is useful for assessing risk factors for infection.
- Aggregate virulence factor scores were lowest among isolates in phylogenetic group B1.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, extended-spectrum ß-lactamase (ESBL)-producing *Escherichia coli* isolates recovered from the following sources were characterized with regard to the occurrence and distribution of uropathogenic and enteric pathogenic virulence factors: surface waters (rivers and lakes, n = 60), the intestines of freshwater fish (n = 33), fresh vegetables (n = 26), retail poultry meat (n = 13) and the fecal samples of livestock (n = 28), healthy humans (n = 34) and primary care patients (n = 13). Among the 207 isolates, 82% tested positive by PCR for one or more of the virulence factors (VF) that predict uropathogenicity, *TraT*, *fyuA*, *chuA*, PAI, *yfcv* or *vat*. Uropathogenic *E. coli* (UPEC) were detected in each of the analyzed sources. Regarding virulence factors for intestinal pathogenic *E. coli*, these were found more rarely and predominantly associated with the aquatic environment, with *aagR* (EAEC) found in isolates from surface waters and STp (porcine heat stable enterotoxin) and LT (heat-labile enterotoxin) associated with isolates from fish. Aggregate VF scores (the number of unique virulence factors detected for each isolate) were lowest among isolates belonging to phylogenetic group B1 and highest among group B2. Clustering of the isolates by phylogenetic group, multilocus sequence type (MLST) and ESBL-types revealed clonal overlaps of A:ST10(CTX-M-1) and D:ST350(CTX-M-1) between the sources of livestock, poultry meat and healthy humans, suggesting livestock, in particular poultry, represents a potential reservoir for these particular UPEC clones. The clones A:ST10(CTX-M-55) and B2:ST131(CTX-M-27), harboring uropathogenic virulence factors were significantly associated with fresh vegetables and with fish, respectively.

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Further clonal complexes with source overlaps included D:ST38(CTX-M-14), D:ST69(CTX-M-15), D:ST405(CTX-M-15) and D:ST648(CTX-M-15), which were found in surface water and healthy humans. Identifying potential reservoirs of UPEC in the environment, animals, food and humans is important in order to assess routes of transmission and risk factors for acquiring UPEC.

1. Introduction

Escherichia coli is a bacterial species of multitudinous characteristics that occurs naturally in the digestive tract of humans and warm-blooded animals. Apart from non-pathogenic commensal isolates, two subdivisions of *E. coli* are, by virtue of their acquisition of virulence factors (VF), etiological agents of intestinal or extraintestinal diseases.

One first major group of pathogenic *E. coli* causes characteristic symptoms of gastrointestinal disease and consists of the pathotypes enteropathogenic *E. coli* (EPEC), shiga toxin-producing *E. coli* (STEC) and its subgroup enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusively adhesive *E. coli* (DAEC) (Nataro and Kaper, 1998). A second major group of pathogenic *E. coli* cause infections outside the gastrointestinal system and are termed extraintestinal pathogenic *E. coli* (ExPEC). This group includes avian pathogenic *E. coli* (APEC), which causes respiratory tract infections and septicemia in poultry and uropathogenic *E. coli* (UPEC) (Kaper et al., 2004). Principally, the human intestinal tract is thought to be the primary reservoir for UPEC from where it can disseminate to the urogenital tract, causing in an ascending manner, urinary tract infections (UTIs) (Pitout, 2012; Singer, 2015).

Virulence factors are distributed unequally among commensal and pathogenic *E. coli*, enabling a classification according to phylogenetic group (Clermont et al., 2000). Thereby, most commensal stains belong to phylogenetic group A or B1, and extraintestinal pathogenic strains, which possess more VF than commensal strains, are assigned to phylogenetic group B2 or D. Whereas for enteropathogenic *E. coli* each pathotype can be characterized and related to disease symptoms by its specific combination of VFs (Kaper et al., 2004), there exists to date no concrete set of virulence factors for defining an *E. coli* as ExPEC or for distinguishing ExPEC subgroups from one another (Singer, 2015). Although a basic virulence gene profile exists for both UPEC and APEC, VFs that are specific to UPEC and that can clearly distinguish it from APEC have not yet been identified (Wiles et al., 2008). Some studies therefore state that some pathogenic as well as non-pathogenic strains in domestic bird populations represent potential UPEC strains in humans (Danzeisen et al., 2013; Johnson et al., 2003; Maluta et al., 2014).

UTIs are among the most frequent human bacterial infections, and constitute a major global burden of disease (Marrs et al., 2005; Totsika et al., 2012). Consequently, the emergence during the last two decades of UPEC harboring antimicrobial resistance genes is a particular threat to human health (Pitout, 2012). Many multidrug resistant *E. coli* strains that are commonly isolated from UTIs belong to specific worldwide endemic clones and have been detected in surface waters and water-related environments (Amos et al., 2014; Tausova et al., 2012; Zurfluh et al., 2013a, 2013b). These clones include the multidrug resistant, extended-spectrum ß-lactamase (ESBL)-producing *E. coli* B2:ST131 or the trimethoprim-sulfamethoxazole resistant *E. coli* Clonal Group A (CGA), a clone that clusters within phylogroup D: ST69 (Totsika et al., 2012). Identifying further potential reservoirs of these and other virulent ExPEC clones may help understand the way they spread throughout the environment.

The purpose of this study was to determine the occurrence and distribution of virulence genes in a collection of ESBL-producing *E. coli* isolates originating from a broad range of environmental, food, animal and human sources. These sources included rivers, lakes, freshwater fish, vegetables, livestock, retail chicken meat, healthy humans and primary care patients.

2. Materials and methods

2.1. Strain collection

The collection of ESBL-producing strains consisted of 60 isolates from rivers and lakes in Switzerland (Zurfluh et al., 2013a, 2013b); 33 strains from the intestines of freshwater fish (Abgottspon et al., 2014a); 26 isolates from different types of fresh vegetables (basil, beans, bitter cucumber cha-om, coriander, chili, curry leaves and okra) imported to Switzerland from the Dominican Republic, India, Thailand and Vietnam (Zurfluh et al., 2015); isolates from fecal samples of chicken (n = 6), pigs (n = 3), lamb (n = 1) and cattle (n = 1) collected from healthy animals entering the slaughterhouses (Geser et al., 2012a); 17 samples originating from a longitudinal sampling study at 3 different broiler chicken farms distributed throughout Switzerland (Zurfluh et al., 2014a, 2014b); strains obtained from poultry meat (n = 13) (Abgottspon et al., 2014b); strains originating from fecal samples of healthy humans (n = 34) or from fecal swabs of primary care patients (n = 13) in Switzerland (Geser et al., 2012b; Nüesch-Inderbinen et al., 2013a). Sources and identities of all strains, as well as isolation dates are indicated in Fig. S1.

2.2. Virulence factor genes

DNA from *E. coli* isolates was extracted by a standard boiling procedure and all 207 isolates were screened by PCR for six markers of virulence associated with UPEC and eight marker genes for IPEC. The UPEC marker genes and the EAEC-specific gene *aggR* were amplified by conventional PCR using primers and conditions described previously for *traT*, *fyuA* and PAI (Johnson and Stell, 2000), *chuA* and *yfcv* (Spurbeck et al., 2012), *vat* (Ewers et al., 2005) and *aggR* (Boisen et al., 2012), respectively.

The IPEC virulence factors *eae* (EPEC), STh, STp and LT (ETEC), *stx1* and *stx2* (STEC) and *ipaH* (EIEC) were detected by real time multiplex PCR (Light Cycler) using QuantiFast Multiplex PCR Kit (Qiagen, Hombrechtikon, Switzerland), and primers and cycling conditions according to the guidelines of the European Union Reference Laboratory for *E. coli* (EU Reference Laboratory for *E. coli*, 2013; http://www.iss.it/vtec/index.php?lang=2&anno=2015&tipo=3).

The aggregate VF score was defined as the number of unique UPEC-VF detected for each isolate, counting the PAI marker as one. Such molecular characteristics predict the extraintestinal virulence potential of an *E. coli* isolate in vivo (Johnson et al., 2006).

2.3. Multilocus sequence typing

For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were amplified by PCR from DNA, as described by Wirth et al. (Wirth et al., 2006). Sequencing of the amplification products was performed by Microsynth (Balgach). Sequences were imported into the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli) to determine MLST types.

Alleles and STs that had not been previously described were assigned new designations by the curators of the database.