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Short communication

ESBL-producing uropathogenic *Escherichia coli* isolated from dogs and cats in Switzerland

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ABSTRACT

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia* (*E.*) *coli* have emerged in human and veterinary medicine. The aim of this study was to investigate the presence of ESBL-producers among uropathogenic *E. coli* isolated from dogs and cats and to characterize detected ESBL-producing isolates by antibiotic susceptibility testing, identification of ESBL genes, multi-locus sequence typing (MLST), detection of putative virulence genes, and analysis of *E. coli* phylogroups. Among the 107 *E. coli* isolates derived from urinary samples (59 from dogs, 40 from cats), eight isolates from four different animals (two dogs, two cats) were found to be ESBL-producers. These isolates were of ST533/CTX-M-15/TEM/phylogroup B1 (four strains from one dog), ST410/CTX-M-15/TEM/phylogroup A (three strains, one from a dog and two from a cat), and ST648/CTX-M-15/phylogroup D (one strain from a cat). In terms of putative virulence factors, all isolates harbored *lpf*A, *sat*, and *tsh*, whereas *iss* was only detected in strains of ST533. Thus, ESBL-producers were detected among uropathogenic *E. coli* from Swiss companion animals and the eight CTX-M-15-producing isolates belonged to three sequence types (ST410, ST533, ST648) and three *E. coli* phylogroups (A, B1, D). For the first time, *E. coli* of ST533 carrying $bla_{CTX\text{-}M\text{-}15}$ were thereby detected in a dog.

1. Introduction

One of the currently most important resistance mechanisms in *Enterobacteriaceae* is based on plasmid-mediated production of enzymes, which inactivate β-lactam-antibiotics including cephalosporins and monobactams by hydrolyzing their β-lactam ring. These enzymes are called extended-spectrum β-lactamases (ESBL) and in addition to the originally known derivates of TEM and SHV β-lactamase families, CTX-M, PER and KPC β-lactamases are now increasingly described (Coque et al., 2008; Bush and Jacoby, 2010). According to recent studies, CTX-M-15 β-lactamase is frequently found in humans and animals (Ewers et al., 2012). ESBLs are inhibited by clavulanic acid, sulbactam, and tazobactam (Bush and Jacoby, 2010), a feature that is used as a criterion for classification of β-lactamases and for ESBL detection. As an additional matter of concern, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like fluoroquinolones, aminoglycosides, and sulfmethoxazole/trimethoprim (Coque et al., 2008).

Since the first description of ESBL-producing *Enterobacteriaceae* from hospitalized humans, ESBL-producing *Escherichia* (*E.*) *coli* have been reported in nosocomial and community-associated infections worldwide. In animals, the first ESBL-producing *E. coli* were detected in fecal samples of a laboratory dog (Matsumoto et al., 1988). Apart from being commensals and playing an important role as intestinal pathogens, *E. coli* are among the most common causative agents for urinary tract infections (UTI).

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ESBL-producing *E. coli* have thereby been described to cause UTI in both humans (Minarini et al., 2007; Bourjilat et al., 2011) and animals (Ewers et al., 2010; O'Keefe et al., 2010; Dierikx et al., 2012). The aim of the present study was therefore (i) to assess the antibiotic susceptibility of uropathogenic *E. coli* isolates obtained throughout 2010 to 2011 from Swiss dogs and cats, (ii) to detect ESBL-producers within this strain collection, and (iii) to characterize ESBL-producing isolates by antibiotic susceptibility testing, identification of ESBL genes, multi-locus sequence typing (MLST), detection of virulence genes, and analysis of *E. coli* phylogroups.

2. Materials and methods

2.1. Bacterial isolates

Between March 2010 and December 2011, a total of 107 *E. coli* strains (59 from dogs, 40 from cats) were isolated from urinary samples of companion animals admitted to the clinic of small animals at University of Zurich, Switzerland. Strains included in this study originated from animals suffering from acute or chronic cystitis. For isolation of these strains, urine samples (obtained by cystocentesis) were plated onto Columbia sheep blood and Gassner agar (Oxoid AG, Pratteln, Switzerland) and incubated overnight at 37 °C. Presumptive *E. coli* isolates were confirmed by biochemical reactions and the automated system VITEK® 2 Compact with ID GN cards (Biomérieux, Marcy l'Etoile, France). Isolates were stored at −20 °C in cryoprotective media until further analyses.

2.2. Antibiotic susceptibility and phenotypic ESBL detection

All 107 *E. coli* isolates were tested for their antimicrobial susceptibility profile using VITEK® 2 Compact system with AST GN cards (Biomérieux) according to the manufacturer's instructions. Isolates suspicious for ESBL-production were subjected to confirmatory tests using Etest® stripes (Biomérieux) containing cefepime, cefotaxime, and ceftazidime, each alone and in combination with clavulanic acid. Additional susceptibility testing of ESBL-producing *E. coli* isolates was performed by disc diffusion (Becton Dickinson AG, Allschwil, Switzerland) using cefotaxim (30 μg), cefalotin (30 μg), ciprofloxacin (5 μg), kanamycin (30 μg), nalidixic acid (30 μg), and streptomycin (10 μg). Results of the antibiotic susceptibility testing (VITEK®, Etest®, and disc diffusion) were interpreted according to the CLSI guidelines for veterinary pathogens if breakpoints were available (CLSI, 2008a) and otherwise (cefotaxime, ciprofloxacin, nalidixic acid, and streptomycin) according to the CLSI guidelines for human pathogens (CLSI, 2008b). Intermediate results were considered resistant.

2.3. Characterization of β-lactamases

Bacterial strains confirmed for producing ESBLs were further analyzed by PCR and by sequencing the whole open reading frames (ORF) of *bla* genes. After DNA extraction using a standard heat lysis protocol, specific primer sets (Microsynth, Balgach, Switzerland) were used to detect β-lactamase-encoding genes belonging to bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$. Detection of group 1, 2, and 9 $bla_{CTX\text{-}M}$ genes was performed according to Woodford et al. (2006). For detection of bla_{SHV} and bla_{TEM}, the protocol of Pitout et al. (1998) was followed, apart from primers for bla_{TEM} (5′-TTC TTG AAG ACG AAA GGG C-3′ and 5′-ACG CTC AGT GGA ACG AAA AC-3′). Sequencing of the whole open reading frames (ORF) of *bla* genes has been previously described (Geser et al., 2012). After purifying resulting amplicons (PCR Purification Kit; QIAGEN, Courtaboeuf, France), custom-sequencing was performed by Microsynth and the nucleotide and protein sequences were analyzed with Codon Code Aligner V. 3.7.1.1. For database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was used.

2.4. Multi-locus sequence typing of ESBL-producers

Internal fragments of seven housekeeping genes (*adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A, and *rec*A) were sequenced (Wirth et al., 2006) and alleles and sequence types (ST) were assigned in accordance with the *E. coli* MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

2.5. Virulence factors in ESBL-producers

Various virulence factors influence the ability of uropathogenic *E. coli* to cause UTI (Pitout, 2012). In this study, we investigated the occurrence of genes for putative virulence factors mediating adhesion (adhesion siderophore *iha*, long polar fimbriae *lpf*A, S fimbrial adhesin *sfa*S, and temperature sensitive hemagglutinin *tsh*), increasing iron-uptake (siderophore for iron *iro*N), and of genes encoding toxins (secreted autotransporter toxin *sat*, serine protease *pic*, vacuolating toxin *vat*, and cytotoxic necrotizing factor *cnf*1). An ArrayTube-based DNA microarray approach (Clondiag Chip Technologies, Jena, Germany) was used according to the manufacturer's instructions.

2.6. Determination of E. coli phylogroups in ESBL-producers

Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogroups (A, B1, B2, and D), in which groups A and B1 typically contain commensal isolates and isolates of groups B2 and D are considered to be more likely carrying pathogenicity-associated genes (Clermont et al., 2000). After DNA extraction using a standard heat lysis protocol, ESBL-producing *E. coli* isolates were assigned to phylogroups according to Clermont et al. (2000).

3. Results and discussion

Among the 107 analyzed *E. coli* isolates derived from urinary samples (59 from dogs, 40 from cats), eight ESBL-producing strains were detected from four different animals (dogs A and B, cat C and D). Dog A is a male cross-breed, dog B a female long-haired dachshund and both cats C and D are male European shorthair cats. From dog A, *E. coli* strains showing the same characteristics in