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Discovery of extended-spectrum β-lactamase producing *Escherichia coli* among hunted deer, chamois and ibex

R. Stephan, H. Hächler

Institute for Food Safety and Hygiene, University of Zurich

Summary

The aim of the present study was to assess for the first time the dissemination of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* in the wild animal ecosystem in Switzerland. Fecal samples of 84 red deer, 64 roe deer, 64 chamois, and 27 ibex were investigated. One sample from a roe deer tested positive for ESBL-producing *E. coli*. The isolate harboured $bla_{CTX\text{-}M\text{-}1}$ and tested negative for both bla_{TEM} and bla_{SHV}. Based on these results low occurrence of ESBL-producing *Enterobacteriaceae* in the wild animal ecosystem in Switzerland must currently be postulated. Further studies are necessary to assess future trends.

Keywords: occurrence, ESBL, hunted wild ruminants

Nachweis von Extended-Spectrum β-Laktamase (ESBL)-produzierenden *E. coli* bei erlegten Rothirschen, Rehen, Gämsen und Steinböcken

Ziel der vorliegenden Studie war eine erstmalige Erhebung zur Verbreitung von Extended-Spectrum β-Laktamase (ESBL)-produzierenden *Escherichia coli* in Ökosystemen mit Wildtier-Populationen in der Schweiz. Kotproben von 84 Rothirschen, 64 Rehen, 64 Gämsen und 27 Steinböcken wurden analysiert. Eine Probe von einem Reh war positiv hinsichtlich ESBL-produzierender *E. coli*. Das Isolat enthielt das Gen $bla_{CTX\text{-}M\text{-}1}$, jedoch weder bla_{TEM} noch bla_{SHV} Gene. Basierend auf diesen Resultaten muss gegenwärtig in Wildtier-Ökosystemen der Schweiz mit einer niedrigen Prävalenz ESBL-produzierender *Enterobacteriaceae* gerechnet werden. Weitere Studien sind nötig, um künftige Trends zu erfassen.

Schlüsselwörter: Vorkommen, ESBL, Wildwiederkäuer, Jagd

Introduction

One of the currently most important resistance mechanisms in *Enterbacteriaceae*, which reduces the efficacy even of modern expanded-spectrum cephalosporins (except cephamycins and carbapenems) and monobactams is based on plasmid-mediated production of enzymes that inactivate these compounds by hydrolyzing their β-lactam ring. Such resistance is encoded by an increasing number of different point-mutational variants, called extended spectrum β-lactamases (ESBL), of classical broad-spectrum β-lactamases (BSBL): most are derivates of TEM and SHV β-lactamase families, whereas other groups, such as CTX-M, OXA, and PER β-lactamases have been described more recently (Coque et al., 2008). The phenotypical difference between BSBLs and ESBLs is that the latter efficiently hydrolyze 3rd- and 4th-generation cephalosporins, additionally to penicillins and lower generation cephalosporins as the BSBLs are capable of. Like BSBLs, ESBLs are inhibited by clavulanic acid, sulbactam and tazobactam (Bradford, 2001), a feature that is used (i) as a criterion for classification of β-lactamases and (ii) for diagnostic ESBL detection purposes. As a matter of growing concern, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like, for example, fluoroquinolones, aminoglycosides and trimethoprim-sulfmethoxazole (Cantòn and Coque, 2006; Gniadkowski, 2001).

Since the first description of ESBL-producing *Enterobacteriaceae* isolated from hospitalized humans (Knothe et al., 1983), many nosocomial outbreaks have been reported. However, since a few years, there is also an in-

crease in the detection of ESBL-producing strains in the community (Mesa et al., 2006; Geser et al., 2012). More recently, several reports have alerted about the dissemination of ESBL-producing *E. coli* into healthy farm animals in several European countries and in Switzerland (for example Duan et al., 2006; Meunier et al., 2006; Geser et al., 2011). The aim of the present study was to assess for the first time the occurrence of ESBL-induced resistance in the wild animal ecosystem in Switzerland by determing the prevalence of ESBL-producing *E. coli* in hunted wild ruminants.

Material and Methods

Sampling

This study was based on investigations carried out during three months of the hunting season 2011 (September–November 2011). Samples originated from shot red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), and ibex (*Capra ibex*). Sampled animals were hunted in the central and eastern part of Switzerland.
Fecal samples (n = 239) originated from 84 red deer, 64 roe deer, 64 chamois, and 27 ibex. State gamekeepers and hunters collected the samples in the field immediately after shooting and evisceration of the wild ruminants. After opening of the large intestine, fecal matter (at least 10 g) was collected from the colon, placed into sterile tubes, and stored frozen (18 °C) by the hunters. For each animal, sex, age, and location of hunting were recorded.

Microbiological analysis

Each sample (about 3 g) was incubated for 24 hours at 37 °C in 30 ml EE Broth (BD, Franklin Lakes, USA) for enrichment. The enriched faecal samples (10 µl) were inoculated onto Brilliance ESBL agar (Oxoid, Hampshire, UK) and incubated at 37 °C for 24 hours under aerobic conditions. For one sample presumptive positive colonies on Brilliance ESBL agar were found. One presumptive positive colony was selected and subcultured onto Triple Sugar Iron (TSI) agar (BD, Franklin Lakes, USA) at 37 °C for 24 hours. The isolate was thereafter subjected to identification by API ID 32 E (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing and ESBL detection

The isolated strain was subjected to susceptibility testing for 17 antimicrobial agents by the CLSI-recommended disc diffusion method and evaluated according to CLSI criteria (Clinical and Laboratory Standards Institute, 2008). The antibiotics tested were: ampicillin (AM) amoxicillin/clavulanic acid (AMC), cefpodoxime (CPD), cephalothin (CF), cefepime (FEP), cefoxitin (FOX), cefotaxime (CTX), cefuroxime (CXM), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GM), streptomycin (S), trimethoprim-sulfamethoxazole (SXT), nalidixic acid (NA), tetracycline (TE), polymyxin B (PB) and imipenem (IMP) (Becton Dickinson, Heidelberg, Germany). The AMC acid disc was placed between the CPD and the CAZ discs and synergy effects were documented. The strain, which showed a synergy effect, was then confirmed as ESBL producer on Mueller-Hinton agar plates using E-Test-ESBL strips containing cefotaxime, cefepime or ceftazidime alone and in combination with clavulanic acid (bioMérieux, Marcy l'Etoile, France).

Identification of β-lactamases

The bacterial strain confirmed for production of ESBLs was further analysed by PCR and sequencing of the whole open reading frames (ORF) of *bla* genes. DNA was extracted by a standard heat lysis protocol. Thereafter, specific primer sets (custom-synthesized by Microsynth, Balgach, Switzerland) were used to search for β-lactamase- encoding genes belonging to bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ (Geser et al., 2012). Resulting amplicons were purified using the PCR Purification Kit (QIAGEN, Courtaboeuf, France) according to the manufacturer's recommendations. Custom-sequencing was performed by Microsynth (Balgach, Switzerland) 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.

Results and Discussion

Fecal samples of 84 red deer, 64 roe deer, 64 chamois, and 27 ibex were investigated to determine the occurrence of ESBL-producing *Enterobacteriaceae* in wild ruminants in Switzerland. One sample from a roe deer (male, 4.5 years old; hunted in the central part of Switzerland, Rotkreuz) tested positive for ESBL-producing *E. coli*. The isolate was positive for $bla_{CTX\text{-}M\text{-}1}$ and negative for both β-lactamase gene families bla_{TEM} and bla_{SHV}. Its full antibiogram was as follows (antibiotic / inhibition zone diameter [mm] / CLSI interpretation [S = susceptible, I = intermediate, R = resistant, S→R = susceptible, but to be reported as resistant]): AM/6/R, AMC/12/R, CF/6/R, CXM/6/R, FOX/19/S, CPD/6/R, CTX/12/R, CAZ/22/S→R, FEP/18/S→R, IMP/30/S, CIP/30/S, NA/25/S, GM/20/S, S/18/S, SXT/21/S, TE/6/R, PB/14/S. The isolate had an additional resistance against tetracycline. This is in accordance with previous reports that ESBL-producing *Enterobacteriaceae* are often co-resistant to other classes of antibiotics like, for example, fluoroquinolones, aminoglycosides and trimethoprim-sulfmethoxazole (Cantòn and Coque, 2006; Gniadkowski, 2001).
There are several studies describing the prevalence and characteristics of ESBL-producing *Enterobacteriaceae* in

wild animals (for an overview see Guenther et al., 2011). In some studies, which were performed in different countries on wild birds, low prevalence of ESBL-producing *E. coli* were found (Bonnedahl et al., 2010; Literak et al., 2010a; Silva et al., 2011; Goncalves et al., 2012). Nevertheless, all these studies show, that ESBL-producing *E. coli* have already been spread into wild ecosystems. Another study done in Poland reported a prevalence of 2% for ESBL-producing *E. coli* in wild boars (Literak et al., 2010b). So far only one study was done on wild ruminants in Portugal (Costa et al., 2006). These authors have tested 3 deer and found two of three animals positive for ESBL-producing *E. coli* (TEM-52).

In summary, based on the results of this study low occurrence of ESBL-producing *Enterobacteriaceae* in the wild animal ecosystem in Switzerland currently has to be postulated. However, further studies are necessary to assess future trends.

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Mise en évidence d'*E. coli* produisant de l'extended-spectrum β-laktamase (ESBL) chez des cerfs, des chevreuils des chamois et des bouquetins abattus à la chasse

Le but de la présente étude était un premier relevé de l'extension des *Escherichia coli* produisant de l'extended-spectrum β-laktamase (ESBL) dans l'écosystème de la population de gibier en Suisse. Des échantillons de selles de 84 cerfs, 64 chevreuils, 64 chamois et 27 bouquetins ont été analysés. Un échantillon provenant d'un chevreuil était positif quant à la présence d'*E. coli* produisant de l'ESBL. L'isolat contenait le gène *bla*$_{CTX\text{-}M\text{-}1}$, mais ni le gène *bla*$_{TEM}$ ni le *bla*$_{SHV}$. Basé sur ces résultats, on peut conclure actuellement à une prévalence réduite d'*Enterobacteriacées* produisant de l'ESBL dans l'écosystème du gibier en Suisse. D'autres études seront nécessaires pour surveiller l'évolution.

Rilevamento in cervi, caprioli, camosci e stambecchi abbattuti di *E. coli* produttori di β-lattamasi a spettro esteso (ESBL)

Lo scopo di questo studio è stato di accertare una prima diffusione di Escherichia coli produttori di β-lattamasi a spettro esteso (ESBL) negli ecosistemi con popolazioni selvatiche in Svizzera. I campioni fecali di 84 cervi, 64 caprioli, 64 camosci e 27 stambecchi sono stati analizzati. Un campione prelevato da un cervo è risultato positivo a *E. coli* produttore di ESBL. L'isolato conteneva il gene *blah*$_{CTX\text{-}M\text{-}1}$, ma non i geni *blah*$_{TEM}$ né *blah*$_{SHV}$. Sulla base di questi risultati bisogna contare sul fatto che attualmente negli ecosistemi selvatici in Svizzera si ha una bassa prevalenza di *Enterobacteriaceae* produttori di ESBL. Ulteriori studi sono necessari per individuare le tendenze future.

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Corresponding author

Roger Stephan
Institute for Food Safety and Hygiene
Vetsuisse Faculty University of Zurich
Winterthurerstr. 272
CH-8057 Zurich
stephanr@fsafety.uzh.ch

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