High prevalence of beta-lactamase-producing Enterobacteriaceae: also in captivity animals from zoological gardens in Belgium

Luc Bauwens1, Pierre Bogaerts*,2, Youri Glupczynski3, Francis Vercammen1

1Royal Zoological Society of Antwerp, Antwerp, Belgium
2Chu Dinant-Godinne Ucl Namur, National Reference Center for Antimicrobial Resistance in Gram, Yvoir, Belgium
3Chu Dinant-Godinne Ucl Namur, National Reference Center for Antimicrobial Resistance in Gram-, Yvoir, Belgium

Background: Over the last decade, there has been a worldwide increase in the occurrence of extended-spectrum β-lactamases (ESBLs) and of plasmidic-AmpC β-lactamases (pAmpCs) which account as major causes of antibiotic resistance. There are only scarce data on the prevalence of the distribution of these resistance mechanisms in zoo animals.

Material/methods: From September 2014 to July 2015, 389 faecal samples were collected from various animals at Antwerp Zoo and Wild Animal Park Planckendael (mammals [n=184], birds [n=167] and reptiles [n=38]). In search for possible reservoirs, 120 animal source food items were also examined.

The samples were cultured on MacConkey agar plus cefotaxime disc after enrichment in broth containing 1µg/ml cefotaxime. Cefotaxime-resistant colonies were identified and subjected to antimicrobial susceptibility testing by disc diffusion according to CLSI standards. Multiplex PCRs targeting ESBLs and pAmpCs genes were performed by in house multiplex PCR. A subset of strains was typed by MLST and assigned by PCR to phylogenetic groups A, B1, B2, C, D, E or F along with sequencing of the ESBL/pAmpC genes.

Results: A total of 96 ESBL/pAmpC-producing Enterobacteriaceae isolated from 90 of the 389 (23.1%) faecal samples were confirmed by multiplex PCR (95 E. coli, 1 Klebsiella pneumoniae). They originated from 41 (22.3%) mammals, 46 (27.5%) birds and 3 (7.9%) reptiles. At Antwerp and at Planckendael respectively, the ESBL/pAmpC isolation rates were similar in the mammals (22.4% vs 22.1%), but markedly different in the birds (15.1% vs 45.6%). Fifty E. coli strains were further characterized: the most frequently detected genes were CTX-M of group 1 (84%; 42/50) including 27 CTX-M-1, 7 CTX-M-15 and 8 CTX-M-32. TEM-52 (n=2), CTX-M-2 and CTX-M-1 + CTX-M-14 (each n=1) and pAmpCs (3 CMY-2 and 1 DHA-1) were also observed. Thirty-six out of 50 (72%) belong to commensal phylogenetic groups A and B1 and C, while 14 (28%) belongs to hypervirulent phylogroup (E, F and D). E. coli isolates belonged to 28 different sequence types (ST). ST131 was not detected. Multidrug resistance (resistance to >3 classes of Abs) was found in 79.2% of the strains.

In food samples, 70% of the sampled 1 day-old chicks carried ST371 CTX-M-1 producing E. coli strains. ST371 was also detected in two CTX-M-1-producing E. coli isolates recovered from 2 prey birds in Antwerp Zoo.
Conclusions: High level (> 20 %) of ESBL/pAmpC-producing E. coli carriage was observed, especially CTX-M G1-producing E. coli. The study of the routes of transmission should be facilitated because of the small, closed and well documented environment of the zoo and the wild park animal. Food and especially one-day chicken could account as among several factors involved in CTX-M-1 acquisition. The possible role of water as source of transmission is currently investigated.