RESEARCH ARTICLE

Extended spectrum beta-lactamase and fluoroquinolone resistance genes and plasmids among *Escherichia coli* isolates from zoo animals, Czech Republic

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Abstract

Commensal *Escherichia coli* isolates from healthy zoo animals kept in Ostrava Zoological Garden, Czech Republic, were investigated to evaluate the dissemination of extended-spectrum beta-lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) genes. A total of 160 faecal samples of various animal species were inoculated onto MacConkey agar with cefotaxime (2 mg L^{-1}) or ciprofloxacin (0.05 mg L^{-1}) to obtain ESBL- or PMQR-positive *E. coli* isolates. Clonality of *E. coli* isolates was investigated by multilocus sequence typing and pulsed-field gel electrophoresis. Plasmids carrying ESBL or PMQR genes were typed by PCR-based replicon typing, plasmid multilocus sequence typing and restriction fragment length polymorphism. Forty-nine (71%, $n = 69$) cefotaxime-resistant and 15 (16%, $n = 94$) ciprofloxacin-resistant *E. coli* isolates harboured ESBL or PMQR genes. Isolates were assigned to 18 sequence types (ST) and 20 clusters according to their macrorestriction patterns by pulsed-field gel electrophoresis. The genes $bla_{CTX\text{-}M\text{-}1}$ and *qnrS1* were detected on highly related IncI1 plasmids assigned to clonal complex 3 (ST3, ST38) and on non-related IncN plasmids of ST1 and ST3, respectively. The gene *qnrS1* was located on related IncX1 plasmids. Dissemination of antibiotic resistance is associated with spreading of particular *E. coli* clones and plasmids of specific incompatibility groups among various animal species.

Introduction

Emergence and dissemination of multidrug-resistant (MDR) gram-negative bacteria is a significant health problem worldwide. The impact of antibiotic resistance is associated with a considerable decrease in the number of efficient antimicrobials available for the treatment of various bacterial infections (Livermore *et al.*, 2007; Strahilevitz *et al.*, 2009; Platell *et al.*, 2011). Cephalosporins and fluoroquinolones belong to critically important antimicrobials in human and veterinary medicine (FAO/WHO/OIE, 2007). The predominant mechanism of resistance to β-lactam antibiotics is mediated by extended-spectrum β-lactamases (ESBL) or AmpC-type β-lactamases (Bergenholtz *et al.*, 2009). Plasmid-mediated quinolone resistance (PMQR) genes confer only a low degree of resistance but their expression can enable development of strains highly resistant to fluoroquinolones (Strahilevitz *et al.*, 2009). The co-localization of ESBL and PMQR genes on the same mobile genetic elements and their association with other resistance genes is of particular concern (Literak *et al.*, 2010; Dolejska *et al.*, 2011a, b).

A number of studies have been focused on MDR *Escherichia coli* derived from humans, wild, domesticated or food animals and from the environment (Literak *et al.*, 2010; Dolejska *et al.*, 2011a, b; Platell *et al.*, 2011).

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Captive animals in zoological gardens can serve as a reservoir of antibiotic-resistant pathogenic bacteria such as *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp., and pathogenic *E. coli* strains (Gopee *et al.*, 2000; Stirling *et al.*, 2008a). A high frequency of resistance including ESBL production and the presence of PMQR genes was found in *E. coli* isolated from zoo animals in Japan and China (Ahmed *et al.*, 2007; Wang *et al.*, 2012).

In this study we performed the detailed genotypic characterization of ESBL- and PMQR-positive *E. coli* isolates from animals kept in a zoo in the Czech Republic over a period of 1 year to assess the potential role of captive animals as a reservoir of emerging MDR bacteria and plasmids carrying antibiotic resistance genes.

Materials and methods

Ostrava Zoological Garden and antibiotic practice

Ostrava Zoological Garden, Czech Republic, houses about 3300 animals of 430 different species including 76 mammalian, 126 avian and 33 reptilian species. It occupies a wooded 1-km^2 site in a suburb of the industrial city of Ostrava in the northeastern part of the Czech Republic. The zoo is visited by about 330 000 people per year.

Antibiotics were administered only on an individual basis under the guidance of the veterinary surgeon for a limited period of < 1–2 weeks in the case of suspected or documented bacterial infections, treatment of bite wounds or as surgical prophylaxis. In the year 2010, when our study was conducted, and during the preceding year, antimicrobials were therapeutically administered to only seven mammalian and two bird species. Antimicrobials used included cefovecin (3rd generation cephalosporin), cefprozil (2nd generation cephalosporin), cefadroxil (1st generation cephalosporin), amoxicillin–clavulanic acid, penicillin, streptomycin, enrofloxacin, marbofloxacin, tetracycline, oxytetracycline, doxycycline and neomycin.

The zoo feeds chickens to 19 animal species including eight mammalian and 11 bird species. All newly-hatched chickens were treated prophylactically with doxycycline for 5 days upon delivery and kept alive in the zoo until being used as feed within 1 month. A limited number of rabbits were also kept in the zoo and fed to zoo animals. The rabbits were treated with trimethoprim–sulphonamide irregularly in cases of digestive disorders. Carnivores were also fed meat from slaughtered food animals not suitable for human consumption. The meat might have contained antibiotic-resistant bacteria and antibiotic residues from recent antibiotic treatments.

Sampling

During the year 2010, Ostrava Zoological Garden was visited four times to obtain faecal specimens of defined individuals of various animal species kept in four distinct geographic areas of the zoo. Within each of four samplings, different animal species occupying a particular geographic area were sampled on one-time basis.

A total number of 160 faecal samples using sterile cotton swabs were obtained from 132 mammalian, avian and reptilian species. From the total of 160 faecal samples, 127 faecal samples were obtained from enclosures that were occupied by a single animal, 21 faecal samples were taken from enclosures occupied by two or more individuals of the same species and 12 faecal samples were taken from enclosures occupied by defined groups of animals belonging to two or more genera. The samples were placed into Amies transport medium (Oxoid, UK) and transported in an isothermal box to a laboratory for further processing. Samples were incubated in buffered peptone water (Oxoid) at 37 °C overnight.

Selective isolation of *E. coli* and antimicrobial susceptibility testing

All samples were enriched in MacConkey (MC) broth (Oxoid) at 37 °C for 24 h and subsequently cultured on MC agar (Oxoid) supplemented with cefotaxime (2 mg L^{-1}) or ciprofloxacin (0.05 mg L^{-1}) at 37 °C overnight. One lactose-positive colony grown on MC agar with antibiotics was selected per one sample and identified using the API 10S test kit (bioMérieux, France) and MALDI-TOF (MALDI Biotyper, Bruker Daltonics). Cefotaxime-resistant *E. coli* isolates were tested using the double-disk synergy test to identify ESBL-production (CLSI, 2008). *Escherichia coli* isolates selected on media with ciprofloxacin were tested for MIC of nalidixic acid and ciprofloxacin using the agar dilution method (CLSI, 2008). Both cefotaxime- and ciprofloxacin-resistant *E. coli* isolates were tested for susceptibility to 12 antimicrobial agents as described previously by Dolejska *et al.* (2011a) according to CLSI guidelines (CLSI, 2008).

Testing of antibiotic resistance genes, integrons and replicons

Genes responsible for ESBL phenotype (bla_{TEM}, bla_{SHV}, bla_{OXA} and bla_{CTX-M}) were identified by PCR and sequencing (Literak *et al.*, 2010). The colonies grown on MCA with ciprofloxacin were tested for PMQR genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, *qepA* and *oqxAB*] by PCR and sequencing (Kim *et al.*, 2009; Literak *et al.*, 2010). All isolates were screened for additional

antibiotic resistance genes and integrons as described previously (Literak *et al.*, 2010). Investigated resistance genes and integrons, primers, conditions of PCR and positive control strains used in the study are listed in Table S1 (available as Supporting Information on the FEMS Microbiology Ecology website). Replicons were tested using PCR-based replicon typing (Carattoli *et al.*, 2005).

Escherichia coli molecular typing methods

All *E. coli* isolates were divided into phylogenetic groups (Clermont *et al.*, 2000). Isolates belonging to phylogenetic groups B2 and D were tested by allele-specific rapid PCR to determine O25b-ST131 *E. coli* clone (Clermont *et al.*, 2009). Epidemiological relatedness of *E. coli* isolates was analysed by XbaI-pulsed field gel electrophoresis (PFGE) (PulseNetUSA, 2004). All macrorestriction profiles were compared using the BIONUMERICS fingerprinting software (Applied Maths, Belgium) dividing the isolates into epidemiologically related clusters (I–XV for cefotaxime- and 1–5 for ciprofloxacin-resistant *E. coli* isolates, respectively) because of a Dice similarity index $\geq$ 85%. The clusters I and V of cefotaxime-resistant isolates and the clusters 1 and 5 of ciprofloxacin-resistant isolates were divided into subgroups on the basis of intraclonal diversity of these clusters (Ia–d, Va–c of cefotaxime- and 1a–b, 5a–b of ciprofloxacin-resistant isolates). Multilocus sequence typing (MLST) was performed according to Wirth *et al.* (2006).

Plasmid characterization

Transferability of plasmids carrying ESBL or PMQR genes was achieved using conjugation and transformation experiments as described elsewhere (Dolejska *et al.*, 2011b). Genomic DNA of transconjugants and transformants was isolated using the boiling technique described by Cattoir *et al.* (2007). Transconjugants and transformants with one plasmid were typed by PCR-based replicon typing (Carattoli *et al.*, 2005), tested for the presence of additional antibiotic resistance genes using PCR (Table S1) and plasmid size was determined using S1-PFGE (Barton *et al.*, 1995). Plasmids were isolated using the alkaline extraction method (Birnboim & Doly, 1979) and compared using restriction fragment length polymorphism performed by EcoRV digestion of plasmid DNA from transformants or transconjugants, dividing them into separate groups based on their restriction patterns. Plasmids showing an identical profile or one band difference were designated with the same letter (e.g. 'A') and those with two to three band differences were designated with a letter and a number of the particular type (e.g. 'A1'). Plasmids belonging to incompatibility groups I1 and N were typed by plasmid multilocus sequence typing (pMLST) (Garcia-Fernandez *et al.*, 2008, 2011) and assigned to sequence types (ST) using the on-line database http://pubmlst.org/plasmid/. Detection of insertion sequences IS*Ecp1* and IS*26* located upstream of $bla_{\text{CTX-M-1}}$ and IS*Ecl2* upstream of the gene *qnrS1* was carried out by PCR mapping (Table S1). Investigation of the $bla_{\text{CTX-M-1}}$ upstream region was conducted as previously described (Dolejska *et al.*, 2011b). The upstream region of *qnrS1* was investigated using forward primer IS*Ecl2* binding to *orfB* of the insertion sequence (Poirel *et al.*, 2007) in combination with *qnrS* reverse primer (Cattoir *et al.*, 2007) targeting a region of *c.* 1.5 kb.

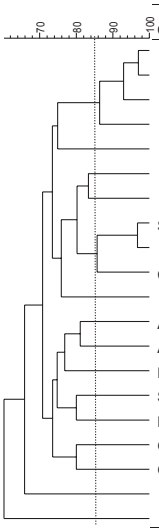
Results

Isolation of ESBL- and PMQR-positive *E. coli* isolates and their antibiotic resistance phenotypes

From the 160 faecal samples of various animal species, 69 (43%) cefotaxime- and 94 (59%) ciprofloxacin-resistant *E. coli* isolates were found. Forty-nine (71%) cefotaxime-resistant and 15 (16%) ciprofloxacin-resistant *E. coli* isolates harboured ESBL or PMQR genes, respectively. All the ESBL-producing *E. coli* isolates were multiresistant (resistant to two or more antibiotic groups). ESBL-positive isolates showed resistance to tetracycline (98%) sulphonamides (96%), nalidixic acid (18%), streptomycin (16%), trimethoprim–sulphamethoxazole and gentamicin (both 4%). Of the PMQR-positive *E. coli* isolates, 12 isolates (80%) were multiresistant. PMQR-positive *E. coli* isolates showed resistance to ampicillin (73%), tetracycline (53%), sulphonamides and trimethoprim–sulphamethoxazole (both 47%), streptomycin (20%), cephalotin and amoxicillin–clavulanic acid (both 13%) and gentamicin (7%). Among the PMQR-positive *E. coli* isolates, MIC of nalidixic acid and ciprofloxacin varied from 8 to $>$ 256 mg L^{-1} and from 0.06 to $>$ 8 mg L^{-1}, respectively.

Resistance genes and integrons detected in ESBL- and PMQR-positive *E. coli* isolates

In the 49 ESBL-producing *E. coli* isolates, the gene $bla_{\text{CTX-M-1}}$ was detected in 48 isolates, and $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-27}}$ in one isolate (130 CTX, Fig. 1). One CTX-M-1-producing isolate (116 CTX) also harboured PMQR gene *qnrS1*. In all PMQR-positive *E. coli* isolates, the gene *qnrS1* was detected (Table 1). The additional antibiotic resistance genes were detected in the ESBL/PMQR-positive isolates (Fig. 1, Table 1): *tet*(A), *tet*(B) encoding resistance to tetracycline, *sul1, sul2* encoding resistance to



Origin	Strain no.	PFGE profile	ST	PG	ESBL *bla*genes	Additional AR genes
Caracal (*Caracal caracal*)	29 CTX	Ia	58	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Eurasian Lynx (*Lynx lynx*)	30 CTX	Ib	58	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
White-tailed Eagle (*Haliaeetusalbicilla*)	73 CTX	Ic	58	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Ural Owl (*Strixuralensis*)	72 CTX	Id	58	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Hyacinth Macaw (*Anodorhynchushyacinthinus*)	116 CTX	II	1324	B2	$bla_{CTX-M-1}$	bla_{TEM-1}, *sul2*, *qnrS1*
Blue Spotted Tree Monitor (*Varanusmacraei*)	130 CTX	III	155	B1	$bla_{CTX-M-15}$, bla_{SHV-27}	*aac(6')-Ib-cr*, bla_{TEM-1}, bla_{OXA-1}, *sul2*, *tet*(A), *tet*(B)
Siberian Crane (*Grusleucogeranus*)	80 CTX	IV	746	A	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Serval (*Leptailurusserval*)	32 CTX	Va	155	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Grey Crowned-crane (*Balearicaregulorum*)	43 CTX	Vb	155	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Common Chimpanzee - group (*Pan troglodytes*)	24 CTX	Vc	847	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Binturong (*Arctictis binturong*)	14z CTX	VI	38	D	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Amur Tiger (*Pantheratigrisaltaica*)	12z CTX	VII	58	B1	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
American Flamingo (*Phoenicopterusruber*)	71 CTX	VIII	1146	A	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Hooded Vulture (*Necrosyrtesmonachus*)	76 CTX	IX	58	A	$bla_{CTX-M-1}$	*sul2*, *tet*(B)
Spur-winged Goose (*Plectropterusgambensis*)	85 CTX	X	48	A	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Eurasian Black Vulture (*Aegypiusmonachus*)	74 CTX	XI	3274	A	$bla_{CTX-M-1}$	*sul2*, *tet*(A)
Grey-headed Swamphen (*Porphyriopoliocephalus*)	86 CTX	XII	2325	A	$bla_{CTX-M-1}$	*sul2*
Great Grey Owl (*Strixnebulosa*)	78 CTX	XIII	1288	A	$bla_{CTX-M-1}$	*tet*(B)
Asian Black Bear (*Ursusthibetanus*)	34 CTX	XIV	410	A	$bla_{CTX-M-1}$	bla_{TEM-1}, *strA*, *sul2*, *tet*(A)
Common Chimpanzee (*Pan troglodytes*)	23 CTX	XV	117	D	$bla_{CTX-M-1}$	*strA*, *sul2*, *tet*(A)

Fig. 1. Characteristics of ESBL-positive *Escherichia coli* isolates from animals in the zoo. AR, antibiotic resistance; PG, phylogenetic group; ST, sequence type. Dendrogram represents diversity of *E. coli* isolates carrying ESBL genes. *Escherichia coli* isolates were divided into clusters (I-XV) according to Dice similarity index of their XbaI macrorestriction profiles ≥ 85%. *Escherichia coli* isolates assigned to the clusters I and V were divided into subgroups (Ia–d, Va–c) owing to intraclonal diversity in these clusters.

Table 1. Characteristic of PMQR-positive *Escherichia coli* isolates from animals in the zoo

Origin	Strain no.	MIC (mg L^{-1})* NAL/CIP	PFGE profile†	ST	PG	PMQR genes	Additional AR genes and integrons
King Vulture (*Sarcoramphus papa*)	143 CIP	> 256/0.25	1a	155	B1	*qnrS1*	–
Amur Tiger (*Panthera tigris*)	13z CIP	> 256/> 8	1b	3275	B1	*qnrS1*	*sul1*, *sul2*, *strA*, *tet*(A),Int1‡
Cottontop Tamarin - group (*Saguinus oedipus*)	3 CIP	> 256/> 8	2	1249	B1	*qnrS1*	*tet*(A)
Serval (*Leptailurus serval*)	32 CIP	8/1	3	1434	A	*qnrS1*	bla_{TEM-32}, *strA*, *tet*(A)
American Flamingo (*Phoenicopterus ruber*)	71 CIP	128/0.25	4	10	A	*qnrS1*	bla_{TEM-1}, *sul2*, *tet*(A)
Southern Screamer (*Chauna torquata*)	101 CIP	16/0.125	5a	1431	B1	*qnrS1*	bla_{TEM-1}, *sul1*, *sul2*, *tet*(A)
Indian Crested Porcupine (*Hystrix indica*)	103 CIP	8/0.5	5b	1431	B1	*qnrS1*	bla_{TEM-1}, *sul1*, *tet*(A)

AR, antibiotic resistance; PG, phylogenetic group; ST, sequence type.

*Minimal inhibitory concentration of NAL, nalidixic acid and CIP, ciprofloxacin to donor strains.

†*Escherichia coli* isolates were divided into clusters (1–5) consisting of clonally related isolates according to Dice similarity index of their XbaI macrorestriction profiles ≥ 85%. *Escherichia coli* isolates assigned to the clusters 1 and 5 were divided into subgroups (1a–b, 5a–b) owing to intraclonal diversity in these clusters.

‡Class 1 integron 1.5 kb: *dfrA1-aadA1*.

sulphonamides, and *strA* encoding resistance to streptomycin. The presence of class 1 integron was detected only in one isolate (13z CIP, Table 1).

Molecular typing of *E. coli* isolates

Forty-nine ESBL-producing *E. coli* isolates were divided into phylogenetic groups A (19 isolates), B1 (18), B2 (1) and D (11). Fifteen PMQR-harbouring *E. coli* isolates belonged to phylogenetic groups A (6) and B1 (9). None of the isolates assigned to phylogenetic groups B2 and D belonged to the O25b-ST131 type.

All *E. coli* were typed using XbaI-PFGE. Sixteen ESBL-producing and four PMQR-positive isolates were non-typeable using this method. Cluster analysis of macrorestriction patterns was performed on 33 ESBL-producing *E. coli* isolates and 11 PMQR-harbouring *E. coli* isolates. For MLST analysis, one to four representative ESBL- and PMQR-positive *E. coli* isolates of each cluster were chosen at random.

ESBL-producing isolates were grouped into 15 clusters (I–XV). Seventy per cent of them belonged to one of five clusters of clonally related isolates. Four of these clusters (I, V, VIII, X) consisted of three to eight isolates that were clonally related. Isolates belonging to the same cluster of clonally related isolates were obtained from different animal species. Ten *E. coli* isolates had unique macrorestriction profiles. Twenty representative ESBL-

positive *E. coli* isolates of each cluster were assigned to 13 different STs (Fig. 1).

Eleven PMQR-harbouring *E. coli* isolates were grouped into five clusters (1–5). In all, 82% of PMQR-positive *E. coli* belonged to one of three clusters of clonally related isolates. Two of these clusters (clusters 2 and 5) consisted of three and four isolates originating from different animal species, respectively. Two PMQR-positive isolates had unique macrorestriction profiles. Seven representative PMQR-positive *E. coli* isolates of each cluster were assigned to six distinct STs (Table 1).

Characterization of plasmids carrying ESBL or PMQR genes

From a total of 33 CTX-M-producing and 11 PMQR-positive isolates, conjugation to *E. coli* or *Salmonella enterica* was demonstrated in 31 and 7, respectively. Only two CTX-M-carrying and none of the PMQR-harbouring plasmids were transferred through transformation. All transformants carried multiple plasmids and these plasmids were therefore not investigated further. For plasmid analysis, one to four transconjugants carrying a single plasmid with ESBL and/or PMQR gene were chosen per one cluster. A total of 14 $bla_{\text{CTX-M-1}}$- and 4 *qnrS1*-carrying plasmids were characterized (Table 2).

Plasmids carrying ESBL or PMQR genes belonging to three incompatibility (Inc) groups IncI1, IncN and IncX1 were identified. The genes $bla_{\text{CTX-M-1}}$ and *qnrS1* were located on IncI1 plasmids varying in size from 100 to 140 kb identified in 13 transconjugants. Plasmids showed related restriction profile (A1–A4) and belonged to clonal complex 3 (CC-3) except for IncI1 plasmid from the isolate 73 CTX, which could not be typed using pMLST. IncN plasmids of ST1 and ST3 carrying $bla_{\text{CTX-M-1}}$ or *qnrS1* genes were found in three transconjugants. Two *qnrS1*-harbouring IncX1 plasmids showed identical restriction profiles. IS*Ecl2* was located upstream of *qnrS1* at the identical distance on all IncX1, IncI1 and IncN plasmids carrying this gene, whereas the gene $bla_{\text{CTX-M-1}}$ was found downstream of the continuous or truncated IS*Ecp1* element (Table 2).

Discussion

Pepperell *et al.* (2002), Lavigne *et al.* (2007) and Cavaco *et al.* (2008) proposed that commensal microflora could represent a long-term reservoir of resistance genes that could be transferred horizontally to other bacteria. In this study, we describe the high prevalence of resistance to third generation cephalosporins and fluoroquinolones in commensal *E. coli* isolates from healthy captive animals in a zoo in the Czech Republic. ESBL-producing and

Table 2. Characteristic of ESBL- and PMQR-positive plasmids from *Escherichia coli* isolates

E. coli isolates			ESBL-/PMQR-positive plasmids						
Strain no.	PFGE profile	ST	Inc group	Size (kb)	RFLP profile*	ST/CC	ESBL-/PMQR-genes	Additional AR genes	Upstream region of $bla_{\text{CTX-M-1}}$/*qnrS1*
73 CTX	Ic	58	I1	100	A1	NT	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
72 CTX	Id	58	I1	100	A1	38/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
71 CTX	VIII	1146	I1	105	A1	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
85 CTX	X	48	I1	105	A1	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
80 CTX	IV	746	I1	100	A2	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
76 CTX	IX	58	I1	100	A2	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2*	IS*Ecp1*
86 CTX	XII	2325	I1	105	A2	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2*	IS*Ecp1*
29 CTX	Ia	58	I1	100	A3	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
30 CTX	Ib	58	I1	100	A3	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
116 CTX	II	1324	I1	100	A3	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, qnrS1*	IS*Ecp1*
12z CTX	VII	58	I1	140	A3	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
74 CTX	XI	3274	I1	100	A3	3/CC-3	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*
71 CIP	4	10	I1	125	A4	38/CC-3	*qnrS1*	$bla_{\text{TEM-1}}$, *sul2*, *tet*(A)	IS*Ecl2*
78 CTX	XIII	1288	N	40	a	1	$bla_{\text{CTX-M-1}}$	–	IS*Ecp1*::IS*26*
34 CTX	XIV	410	N	40	a	1	$bla_{\text{CTX-M-1}}$	*sul2, tet*(A)	IS*Ecp1*::IS*26*
32 CIP	3	1434	N	50	b	3	*qnrS1*	$bla_{\text{TEM-32}}$, *strA, tet*(A)	IS*Ecl2*
101 CIP	5a	1431	X1	50		–	*qnrS1*	$bla_{\text{TEM-1}}$	IS*Ecl2*
13z CIP	1b	3275	X1	50		–	*qnrS1*	–	IS*Ecl2*

AR, antibiotic resistance; CC, clonal complex; Inc, incompatibility group; NT, non-typeable; ST, sequence type.

All plasmids were successfully transferred via conjugation to *E. coli* MT102RN and *Salmonella* Typhimurium SL5325 or *Salmonella* Enteritidis Fa8065 recipient strains.

*RFLP profile determined by EcoRV digestion.

PMQR-harbouring isolates were found in 31% ($n = 160$) and 9% of sampled animals belonging to different avian, mammalian and reptilian species, respectively. Similar results were obtained by Wang *et al.* (2012), who found 32% ($n = 206$) ESBL-producing *E. coli* isolates including strains carrying various PMQR genes in primates kept in six zoos in China.

The high occurrence of ESBL-producing and PMQR-harbouring isolates found in our study could be associated with antibiotic therapy used in animals for treatment of various infections. Beta-lactam antibiotics including the second and the third generation cephalosporins, fluoroquinolones, tetracyclines and aminoglycosides were applied, however, only to limited number of animals. Selection of ESBL-producing *E. coli* in the gut microflora of animals induced by cephalosporin treatment has been demonstrated (Cavaco *et al.*, 2008). Most isolates showed resistance to multiple antibiotics including tetracyclines, sulphonamides, aminoglycosides and quinolones; therefore, the drugs of other antibiotic groups might have played a role in co-selection of ESBL- and PMQR-positive *E. coli* clones or plasmids carrying particular resistance genes.

Several studies have concentrated on the possible transmission of zoonotic ESBL-producing *E. coli* through the food chain (Livermore *et al.*, 2007; Leverstein-van Hall *et al.*, 2011; Platell *et al.*, 2011). In the zoological garden, 19 sampled animals were fed chickens and rabbits treated with antibiotics. These chickens and rabbits are a plausible source of ESBL-producing and PMQR-harbouring *E. coli* isolates found in the zoo animals. *Escherichia coli* clones belonging to particular sequence types (ST10, ST58, ST117) harbouring $bla_{\text{CTX-M-1}}$ or *qnrS1* isolated from different avian and mammalian species kept in the Czech zoo have been identified previously in poultry, poultry meat and humans in the Netherlands (Leverstein-van Hall *et al.*, 2011), supporting our theory of zoonotic transmission of antibiotic-resistant *E. coli* clones via the food chain. However, samples of food (chickens, rabbits or other food sources) and water sources of zoo animals or faecal samples of possible vectors (e.g. zoo-keepers) were not analysed in our study.

Identical or closely related IncI1 plasmids of clonal complex 3 were the most prevalent among $bla_{\text{CTX-M-1}}$-harbouring *E. coli* isolates of different clonal lineages originating from several animal species fed chickens and rabbits. IncI1 plasmids belonging to ST3 carrying $bla_{\text{CTX-M-1}}$ are widely disseminated in *E. coli* and *S. enterica* isolates from poultry throughout Europe (Garcia-Fernandez *et al.*, 2008; Cloeckaert *et al.*, 2010; Leverstein-van Hall *et al.*, 2011) but they have been also found in other food-producing and companion animals as well as humans (Madec *et al.*, 2011). Results of plasmid analysis might indicate that CTX-M-1-positive *E. coli* harbouring IncI1 plasmids isolated from zoo animals could originate in poultry.

Plasmids of incompatibility group IncN harbouring ESBL or PMQR genes identified in our study have been previously demonstrated in *E. coli* and *S. enterica* isolates from humans, animals and the environment in various European countries (http://pubmlst.org/plasmid, Dolejska *et al.*, 2013). IncN plasmids belonging to ST1 harbouring $bla_{\text{CTX-M-1}}$ are broadly disseminated in *E. coli* isolates from poultry, pigs, horses, cattle, wild waterbirds and humans (http://pubmlst.org/plasmid, Dolejska *et al.*, 2013). Plasmids of IncX1 group carrying *qnrS1* genes have been identified in *E. coli* from poultry (Cerquetti *et al.*, 2009), wild waterbirds (Literak *et al.*, 2010), horses (Dolejska *et al.*, 2011a) and humans (Literak *et al.*, 2011).

The abundance of MDR *E. coli* isolates in respective faecal samples of zoo animals was not determined in our study. ESBL- or PMQR-positive *E. coli* might have constituted either the predominant population of *E. coli* in the intestine of particular zoo animals or might have been transient flora. Duval-Iflah *et al.* (1981) showed that indigenous flora that was adapted to host intestine was the dominant population and exerted a barrier against the establishment of externally introduced *E. coli* strains. However, even the transient presence of MDR *E. coli* harbouring conjugative ESBL- or PMQR-carrying plasmids might be of particular concern. Licht *et al.* (1999) showed that conjugation in the intestine of a streptomycin-treated mouse model occurred initially at high rates after introduction of high numbers of the *E. coli* donor cells. Thereafter the rate of horizontal transfer of plasmids dropped but transconjugants remained present in the faeces of investigated mice at rather high levels.

In our study, we showed that zoo animals can represent a reservoir of resistance genes to critically important antimicrobials. Dissemination of these resistance genes is of particular concern. Multiresistance can be transmitted via direct contact between animals and humans, as has been shown by Moodley & Guardabassi (2009) and Dolejska *et al.* (2011a). Moreover, there has been a rise in the popularity of petting farm areas of zoos with direct contact between animals and visitors, especially children (Stirling *et al.*, 2008b).

In conclusion, our study showed a high prevalence of *E. coli* harbouring ESBL or PMQR genes among various animal species in the Czech zoological garden. The extensive distribution of MDR *E. coli* is associated with successful spread of particular clones as well as horizontal gene transfer of related plasmids. Results of molecular analyses might indicate a common source of MDR

E. coli possibly spreading via the food chain. However, further investigation is warranted to assess the potential impact of transmission of MDR *E. coli* via the food chain in dissemination of antimicrobial resistance in zoo animals.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of primers for antibiotic resistance genes and mobile genetic elements used in the study.