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OXA-72-Mediated Carbapenem Resistance in Sequence Type 1 Multidrug (Colistin)-Resistant *Acinetobacter baumannii* Associated with Urinary Tract Infection in a Dog from Serbia

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Multidrug-resistant *Acinetobacter baumannii* is primarily important as a causative agent of difficult-to-treat nosocomial infections in humans (1). *A. baumannii* sporadically causes infections in animals, including dogs (1, 2). Carbapenem-resistant *A. baumannii* harboring bla_{OXA-72} has been first reported in 2017, from a parrot in Luxembourg (2). bla_{OXA-23}-mediated carbapenem-resistant A. *baumannii* has been associated with urinary infection in cats in Germany (3) and Portugal (4), and it was reported from a carrier dog in France (5). The isolation was performed in 2016 from a urine sample taken in a private veterinary clinic by catheterization from the dog with the fever, and it was submitted immediately to the Department of Microbiology, Faculty of Veterinary Medicine, University of Belgrade (FVM-UB), Serbia. The specimen was sampled prior to antibiotic treatment. After the incubation, approximately 60,000 CFU/ml was counted and all CFU showed the same colony morphology. *A. baumannii* was identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics).

The colistin MIC was determined by broth microdilution according to the CLSI standard (6). MIC values of other antibiotics were determined by Etest. Full results are given in Table 1. The strain was resistant to piperacillin and piperacillin-tazobactam (MIC, ≥128 μg/ml); ceftazidime, cefepime, and cefotaxime (MIC, ≥64 μg/ml); imipenem and meropenem (MIC, ≥16 μg/ml); gentamicin and tobramycin (MIC, ≥16 μg/ml); amikacin (MIC, ≥64 μg/ml); ciprofloxacin (MIC, ≥4 μg/ml); trimethoprim-sulfamethoxazole (MIC, ≥320 μg/ml); and colistin (MIC, 16 μg/ml).

Preliminary detection of antibiotic resistance genes was performed using the Carb-Detect AS-2 and PanType AS-2 kits (Alere Technologies, Germany). The gene families that responded positively in the array (with the addition of bla_{ADC}) were further typed by PCR and sequencing using previously described primers (7–18). Genes associated with acquired carbapenemase ($bla_{OXA-40-like}$), chromosomal oxacillinase ($bla_{OXA-51-like}$), and β-lactamase (bla_{TEM}) were detected. DNA sequencing revealed that bla_{OXA-72} acquired carbapenemase belonging to the OXA-24/40 derivate (sequence shared 100% nucleotide similarity with EF534256 and 100% protein similarity with ABP87779 with the already published and curated sequence for bla_{OXA-72} obtained from https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/ [formerly Lahey]). bla_{TEM-1} with a stop codon near its 3′ end was detected.

The IS*Aba1* element upstream of $bla_{OXA-51-like}$ was not found, eliminating overexpression of this mechanism. bla_{ADC} was detected with the IS*Aba1* element upstream, thus explaining the resistance to cephalosporins. The aminoglycoside resistance genes

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TABLE 1 Summary of the MIC values, detected genes, and gene products in *A. baumannii* ST1 (GA60)[a]

Antibiotic(s)	MIC (μg/ml)	Gene(s) detected	Gene product/function	Comment	Reference(s)
Ceftazidime	≥64	bla_{ADC}	Intrinsic chromosomal β-lactamase (*Acinetobacter*-derived cephalosporinase)	IS*Aba1* element upstream of bla_{ADC} detected	12
Cefotaxime	≥64				
Cefepime	≥64				
Imipenem	≥16	$bla_{OXA-40-like}$	Class D (OXA) β-lactamase (carbapenem-hydrolyzing oxacillinase)		8
Meropenem	≥16	bla_{OXA-72}			
		$bla_{OXA-51-like}$	Intrinsic chromosomal oxacillinase (carbapenem-hydrolyzing oxacillinase)	IS*Aba1* was not found, no expression	12
		bla_{TEM-1}	Class A broad-spectrum β-lactamase	Stop codon detected near the 3′ end, no expression	10, 11
Piperacillin	≥128	$bla_{OXA-40-like}$		Generally, OXA enzymes are resistant to inhibition by clavulanate, sulbactam, and tazobactam	20
Piperacillin-tazobactam	≥128	bla_{OXA-72}			
Gentamicin	>16	*aac(3)-Ia*	Aminoglycoside N-acetyltransferase	Resistance to gentamicin	19
Tobramycin	>16				
Amikacin	≥64	*aac(6′)-Ib*	Aminoglycoside N-acetyltransferase	Resistance to tobramycin, amikacin, netilmicin**	
		aadA1, *aadA1a*	Aminoglycoside O-nucleotidyltransferases	Resistance to spectinomycin** and streptomycin**	
		aphA-7	Aminoglycoside O-phosphotransferase	Resistance to kanamycin** and neomycin**	
Trimethoprim-sulfamethoxazole	≥320	*sul1*	Dihydropteroate synthase	Resistance to sulfamethoxazole	***
		dfrA18	Dihydrofolate reductase	Resistance to trimethoprim	
Chloramphenicol	ND	*catA1*	Chloramphenicol acetyltransferase	Resistance to chloramphenicol	***
Tetracyclines	ND	*tet*(A)	Efflux pump	Resistance to tetracycline	***
Ciprofloxacin	≥4	*gyrA*	DNA gyrase A	Mutations in quinolone resistance-determining-region (QRDR) of GyrA Ser83Leu	13
		parC	Topoisomerase IV, subunit A	Mutations in quinolone resistance-determining-region (QRDR) of ParC Ser80Leu	
Colistin	16	*pmrCAB*	Two-component response regulator and sensor kinase PmrA/B, expression of genes implicated in lipid A modification	Colistin mutations in PmrC (R125P, I131V, H499R*), PmrA (A80V), and PmrB (R231T, P360Q*)	7, 15

[a]Abbreviations and symbols: ND, not determined; *, alteration has previously been associated with resistance to colistin; **, not included in this research; ***, included in microarray panel.

aac(3)-Ia, *aac(6′)-Ib*, *aadA1*, and *aphA-7* were detected (19). The resistance to ciprofloxacin was attributed to mutations in the quinolone resistance-determining region (QRDR) of GyrA Ser83Leu and ParC Ser80Leu. Resistance to chloramphenicol was confirmed by detection of *catA1*, resistance to tetracycline was confirmed by detection of *tet*(A), and resistance to trimethoprim-sulfamethoxazole was confirmed by detection of *sul1* and *dfrA18*. Sequencing of *lpx* genes and comparison with colistin-sensitive strain ATCC 19606 revealed that there are no mutations in *lpxA*, *lpxD*, or *lpxC*. In addition, the PmrCAB region contained mutations also in PmrC (R125P, I131V, and H499R*), PmrA (A80V), and PmrB (R231T and P360Q*) (alterations marked with an asterisk have previously been associated with resistance to colistin) (7). The presence of bla_{OXA-72} on a ca.-10-kb plasmid was confirmed by Southern blotting as well as by transformation of meropenem-sensitive and plasmid-free *A. baumannii* BM4547 (kindly provided by L. Poirel and P. Nordmann) using a Gene Pulser II electroporator (Bio-Rad) with standard settings for *Escherichia coli*. bla_{OXA-72}-harboring transformants of BM4547 were grown on agar with 10 μg/ml meropenem. The plasmid was replicon typed (16) and belonged to replicon group GR2, which is associated with plasmid pACICU1 variant Aci1. This plasmid, named pS60, carried neither other β-lactamases, non-β-lactamase genes, nor integrons. A 3,186-bp class 1 integron with gene cassette *aac(6′)-Ib-aac(3)-Ia-gcuP-gcuQ-aadA1a* was detected, and it was not localized on pS60 where bla_{OXA-72} was located. Multilocus sequence typing (MLST) revealed that this strain belonged to sequence type 1 (ST1) (*A. baumannii* MLST databases, https://pubmlst.org/abaumannii/).

In conclusion, bla_{OXA-72}-harboring, colistin-resistant *A. baumannii* in companion animals is exceptionally rare, but it deserves special consideration for both animal and public health due to its resistance to last-resort antibiotics.

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