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SHORT COMMUNICATION

Extensively Drug-resistant *Acinetobacter baumannii* Belonging to International Clone II from A Pet Cat with Urinary Tract Infection; The First Report from Pakistan

ZEESHAN TAJ[1, 2], MUHAMMAD HIDAYAT RASOOL[1], AHMAD ALMATROUDI[3], MUHAMMAD SAQALEIN[1, 2] and MOHSIN KHURSHID[1*]

[1] Department of Microbiology, Government College University Faisalabad, Pakistan
[2] Pet Care Clinic, Faisalabad, Pakistan
[3] Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Qassim, Saudi Arabia

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Abstract

The carbapenem-resistant *Acinetobacter baumannii* (CRAB) has got global attention as a notorious nosocomial pathogen. This study describes a case of urinary tract infection in a 2-years old pet female cat infected with *A. baumannii*. The susceptibility profiling, screening for the resistance determinants, and the multilocus sequence typing was performed. The *A. baumannii* isolate was found to harbor the *bla*OXA23-like gene and corresponded to International clone II that has been widely reported to be involved in human infections. The study proposes that the pets may contribute towards the spread of clinically relevant antimicrobial-resistant pathogens.

K e y w o r d s: MLST, sequence types, carbapenemases, *Acinetobacter baumannii*, companion animals

Acinetobacter baumannii is the most prevalent species of genus Acinetobacter that caused various nosocomial infections in clinical settings. *A. baumannii* is quite ubiquitous and has been found in water, air, and soil. Although the studies related to the animal infections caused by *A. baumannii* are limited, the reports have highlighted the involvement of *Acinetobacter* species in respiratory, urinary, bloodstream, and wound infections with an attributable mortality of 47% in pets (Pomba et al. 2017). The therapeutic management of carbapenem-resistant *A. baumannii* (CRAB) is challenging in clinical medicine (Sohail et al. 2016; Khurshid et al. 2017). The emergence of multidrug-resistant CRAB isolates has been increasingly reported and is mainly associated with the acquisition of the *bla*NDM gene and overexpression of the *bla*OXA-23 gene in bovines and equines (Poirel et al. 2012; Smet et al. 2012; Zhang et al. 2013). However, the majority of carbapenem-resistant phenotypes in *A. baumannii* isolates from the pets are mainly linked with the increased expression of the intrinsic genes (Ewers et al. 2017).

The data regarding the mechanisms underlying the antimicrobial resistance and molecular epidemiology of *Acinetobacter* species from the veterinary origin are limited compared to the *A. baumannii* strains from humans. However, the studies have revealed that the *A. baumannii* isolates from veterinary sources may harbor identical antimicrobial resistant determinants as well as share the identical clonal lineages as human strains suggesting a common source of infection (Zordan et al. 2011; Puntener-Simmen et al. 2019). Here, we have described a CRAB isolate harboring the *bla*OXA-23 gene from a pet cat suffering from urinary tract infection.

A two-years-old pet cat was brought to our pet clinic with dysuria and hematuria. The urine sample was aseptically collected, which showed significant bacteriuria, and *A. baumannii* was solely obtained. The cat was having a history of persistent fever, pyuria, anorexia, weight loss, postural changes, and mood disorders from the last three months, which were previously attempted to treat with multiple courses of antimicrobial agents empirically. Initially, the oral amoxicillin-clavulanate

* Corresponding author: M. Khurshid, Department of Microbiology, Government College University Faisalabad, Pakistan; **e-mails: mohsin.mic@gmail.com, mohsinkhurshid@gcuf.edu.pk**

Table I
Resistance genes detected in the *A. baumannii* strain isolated in a urine sample from the urinary tract infection suffering cat.

Antibiotic category	Mechanism	Resistance associated gene	Resistance phenotypes
Aminoglycosides	16S rRNA methyltransferase genes	*armA*	Amikacin[a], Gentamicin[b], Tobramycin[b]
	Aminoglycoside modifying enzymes	*aphA6*, *aadB*, and *aacC1*	
Carbapenems	Oxacillinases	*blaOXA-23*	Imipenem[c], Meropenem[d], Ceftazidime[e], Cefotaxime[e], Ceftriaxone[e], Cefepime[f], Piperacillin-tazobactam[g], Ampicillin-sulbactam[h]
Fluoroquinolones	Quinolones Resistance Determining Region (QRDR)	*gyrA* gene mutation (Ser83Leu)	Ciprofloxacin[i]
Sulfonamides	Dihydropteroate synthase	*Sul2*	Sulfamethoxazole-Trimethoprim[j]
Tetracyclines	Tetracycline efflux MFS transporter	*tetB*	Doxycycline[k]

[a] MIC 1024 µg/ml, [b] MIC 512 µg/ml, [c] MIC 16 µg/ml, [d] MIC 32 µg/ml, [e] MIC 64 µg/ml, [f] MIC 32 µg/ml, [g] MIC 128/4 µg/ml, [h] MIC 64/32 µg/ml, [I] MIC 16 µg/ml, [j] MIC 16/304 µg/ml, [k] MIC 128 µg/ml

suspension was administered at a dose rate of 62.5 mg/cat PO twice daily for 14 days, followed by ciprofloxacin at a dose rate of 6 mg/kg PO q12h for 10 days.

The *A. baumannii* isolate was identified by amplification of the *recA* gene and ITS region in a multiplex PCR as described previously, as well as the amplification of the *bla*OXA-51 gene (Khurshid et al. 2017; Khurshid et al. 2020). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) according to the CLSI guidelines (CLSI 2015). The genes encoding the carbapenem resistance and the presence of insertion element i.e., IS*Aba1*, were detected using PCR as described previously using specific primers (Khurshid et al. 2017). The PCR was performed to detect the presence of 16S rRNA methyltransferase genes (*arm*A, *rmt*A, *rmt*B, *rmt*C, *rmt*D, and *rmt*E) and aminoglycoside modifying enzymes (AMEs) i.e., *aphA1*, *aphA6*, *aadB*, *aadA1,* and *aacC1* and tetracycline and sulfonamide resistant genes including *tetA*, *tetB*, *sul1*, *sul2*, and *sul3* genes (Khurshid et al. 2019). The isolates were also screened for plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*) as well as mutations in the quinolone resistance-determining region by sequencing *gyrA* and *parC* gene (Gu et al. 2015). The multi-locus sequence typing (MLST) was performed using primers recommended by the MLST database for *A. baumannii* following the Pasteur scheme.

The strain was susceptible only to colistin (MIC 0.5 µg/ml), and tigecycline (MIC 1 µg/ml). The higher MICs of imipenem (MIC 16 µg/ml), meropenem (MIC 32 µg/ml), ceftazidime, cefotaxime, ceftriaxone (MIC 64 µg/ml), cefepime (MIC 32 µg/ml), piperacillin-tazobactam (MIC 128/4 µg/ml), and ampicillin-sulbactam (MIC 64/32 µg/ml) were linked with the production of *bla*OXA-23 (Opazo et al. 2012; Khurshid et al. 2017). The resistance to aminoglycoside i.e., MICs of amikacin (MIC 1024 µg/ml), gentamicin, and tobramycin (MIC 512 µg/ml) was attributed to the presence of 16S rRNA methyltransferase genes i.e., the *armA* gene as well as AMEs i.e., *aphA6*, *aadB*, and *aacC1*. Moreover, the MIC of trimethoprim-sulfamethoxazole was 16/304 µg/ml attributed to the presence of the *sul2* gene. The *A. baumannii* isolates showed resistance to tetracycline/doxycycline with a doxycycline MIC equal to 128 µg/ml, and it was related to the presence of the *tetB* gene. The strain was found resistant to ciprofloxacin (MIC 16 µg/ml), which was attributed to the mutation (Ser83Leu) in the *gyrA* gene. The genes conferring resistance to different antimicrobial agents that were found in the *A. baumannii* strain are summarized in Table I. The IS*Aba1* was found upstream to the *bla*OXA-51 and *bla*OXA-23 genes.

The concerns related to the possible threats of the *bla*OXA-23 harboring CRAB among the pets and other farm animals have been increasing (Ewers et al. 2017). The information on *A. baumannii* in veterinary settings is, however, limited, and data related to the comparison of strains isolated from the humans and veterinary sources are quite inadequate (van der Kolk et al. 2019). From Pakistan, this is the very first report of extensively drug-resistant (XDR) CRAB isolates harboring the acquired the *bla*-OXA-23 and *armA* genes from an infected pet cat, which drives the attention towards the possible transmission of these XDR pathogens from the companion animals to humans.

The *bla*OXA-23 gene is a major cause of carbapenem resistance throughout the world; therefore, it can be considered a virulence marker and is located on the chromosome as well as on the plasmids. Moreover, the studies have found a strong correlation between the occurrence of the *bla*OXA-23 gene and multidrug-resistant phenotypes (Pomba et al. 2014; Zowawi et al. 2015; Khurshid et al. 2017).

The MLST has shown that the *A. baumannii* strain belonged to the sequence type 2 (ST2), and the eBURST analysis has revealed that it corresponded to the inter-

national clonal lineage 2. The study conducted by Tada and his colleagues concluded that there is worldwide dissemination of this clone also harboring the *bla*OXA-23 and *armA* genes but does not suggest the human-to animal transmission (Tada et al. 2015). Notably, the *A. baumannii* ST2 has been extensively isolated from humans, while some of the recent reports have also indicated the presence of ST2 in pets (Puntener-Simmen et al. 2019). The carbapenem-resistant isolates in these studies were found to possess the intrinsic *bla*OXA-51 gene solely or accompanied by the acquired the *bla*OXA-23-like genes. Interestingly, the *A. baumannii* isolates were reported among the pets living in the community (Lupo et al. 2017). Although the data is quite limited regarding the carriage of *Acinetobacter* species beyond the veterinary clinical settings, more than a few studies during the recent few years have detected the *A. baumannii* isolates in the community among domestic birds, dogs, livestock, and other large animals. These studies specify that the incidence of *A. baumannii* infections among animals is increasing and these animals may serve as a reservoir for *A. baumannii*, particularly carbapenem-resistant strains, due to their selective advantage compared to the susceptible strains (Pomba et al. 2014; van der Kolk et al. 2019).

This study has reported an extensively drug-resistant *A. baumannii*, harboring the *bla*OXA-23 gene and other resistant associated genes isolated from a companion animal previously treated with multiple empirical antimicrobial courses. The infected pets may contribute to the pool of multidrug-resistant clinically relevant bacteria and their interaction with the human may transmit these pathogens to humans. The extensive epidemiological studies are essential for a better understanding of the extent of distribution, risk factors, and the directions of transmission of these multidrug-resistant strains.

ORCID

Mohsin Khurshid https://orcid.org/0000-0002-3196-2857

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Conflict of interest

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Literature

CLSI. Performance standards for antimicrobial susceptibility testing. Twenty-Fifth Informational Supplement. Wayne (USA): Clinical and Laboratory Standard Institute; 2015.

Ewers C, Klotz P, Leidner U, Stamm I, Prenger-Berninghoff E, Göttig S, Semmler T, Scheufen S. OXA-23 and IS *Aba1* – OXA-66 class D β-lactamases in *Acinetobacter baumannii* isolates from companion animals. Int J Antimicrob Agents. 2017 Jan;49(1):37–44. https://doi.org/10.1016/j.ijantimicag.2016.09.033

Gu D, Hu Y, Zhou H, Zhang R, Chen GX. Substitutions of Ser83Leu in GyrA and Ser80Leu in ParC associated with quinolone resistance in *Acinetobacter pittii*. Microb Drug Resist. 2015 Jun; 21(3):345–351. https://doi.org/10.1089/mdr.2014.0057

Khurshid M, Rasool MH, Ashfaq UA, Aslam B, Waseem M, Xu Q, Zhang X, Guo Q, & Wang M. Dissemination of bla_{OXA-23} harboring carbapenem-resistant *Acinetobacter baumannii* clones in Pakistan. J Glob Antimicrob Resist. 2020;S2213-7165(2220):30002–30003.

Khurshid M, Rasool MH, Ashfaq UA, Aslam B, Waseem M. Emergence of IS *Aba1* harboring carbapenem-resistant *Acinetobacter baumannii* isolates in Pakistan. Future Microbiol. 2017 Nov; 12(14):1261–1269. https://doi.org/10.2217/fmb-2017-0080

Khurshid M, Rasool MH, Siddique MH, Azeem F, Naeem M, Sohail M, Sarfraz M, Saqalein M, Taj Z, Nisar MA, et al. Molecular mechanisms of antibiotic co-resistance among carbapenem resistant *Acinetobacter baumannii*. J Infect Dev Ctries. 2019 Oct 31;13(10): 899–905. https://doi.org/10.3855/jidc.11410

Lupo A, Châtre P, Ponsin C, Saras E, Boulouis HJ, Keck N, Haenni M, Madec JY. Clonal spread of Acinetobacter baumannii sequence type 25 carrying bla_{OXA-23} in companion animals in France. Antimicrob Agents Chemother. 2017 Jan;61(1):e01881–16. https://doi.org/10.1128/AAC.01881-16

Opazo A, Domínguez M, Bello H, Amyes SGB, González-Rocha G. OXA-type carbapenemases in *Acinetobacter baumannii* in South America. J Infect Dev Ctries. 2011 Dec 24;6(04):311–316. https://doi.org/10.3855/jidc.2310

Poirel L, Berçot B, Millemann Y, Bonnin RA, Pannaux G, Nordmann P. Carbapenemase-producing *Acinetobacter* spp. in cattle, France. Emerg Infect Dis. 2012 Mar;18(3):523–525. https://doi.org/10.3201/eid1803.111330

Pomba C, Endimiani A, Rossano A, Saial D, Couto N, Perreten V. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat. Antimicrob Agents Chemother. 2014 Feb;58(2):1267–1268. https://doi.org/10.1128/AAC.02527-13

Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, et al. Public health risk of antimicrobial resistance transfer from companion animals. J Antimicrob Chemother. 2017 Apr 1;72(4):957–968.

Püntener-Simmen S, Zurfluh K, Schmitt S, Stephan R, Nüesch-Inderbinen M. Phenotypic and genotypic characterization of clinical isolates belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex isolated from animals treated at a veterinary hospital in Switzerland. Front Vet Sci. 2019 Feb 5;6:17. https://doi.org/10.3389/fvets.2019.00017

Smet A, Boyen F, Pasmans F, Butaye P, Martens A, Nemec A, Deschaght P, Vaneechoutte M, Haesebrouck F. OXA-23-producing *Acinetobacter* species from horses: a public health hazard? J Antimicrob Chemother. 2012 Dec 01;67(12):3009–3010. https://doi.org/10.1093/jac/dks311

Sohail M, Rashid A, Aslam B, Waseem M, Shahid M, Akram M, Khurshid M, Rasool MH. Antimicrobial susceptibility of *Acinetobacter* clinical isolates and emerging antibiogram trends for nosocomial infection management. Rev Soc Bras Med Trop. 2016 Jun;49(3):300–304. https://doi.org/10.1590/0037-8682-0111-2016

Tada T, Miyoshi-Akiyama T, Shimada K, Nga TTT, Thu LTA, Son NT, Ohmagari N, Kirikae T. Dissemination of clonal complex 2 *Acinetobacter baumannii* strains co-producing carbapenemases

and 16S rRNA methylase ArmA in Vietnam. BMC Infect Dis. 2015 Dec;15(1):433. https://doi.org/10.1186/s12879-015-1171-x

van der Kolk JH, Endimiani A, Graubner C, Gerber V, Perreten V. *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. J Glob Antimicrob Resist. 2019 Mar; 16:59–71. https://doi.org/10.1016/j.jgar.2018.08.011

Zhang WJ, Lu Z, Schwarz S, Zhang RM, Wang XM, Si W, Yu S, Chen L, Liu S. Complete sequence of the bla_{NDM-1}-carrying plasmid pNDM-AB from *Acinetobacter baumannii* of food animal origin. J Antimicrob Chemother. 2013 Jul;68(7):1681–1682. https://doi.org/10.1093/jac/dkt066

Zordan S, Prenger-Berninghoff E, Weiss R, van der Reijden T, van den Broek P, Baljer G, Dijkshoorn L. Multidrug-resistant *Acinetobacter baumannii* in veterinary clinics, Germany. Emerg Infect Dis. 2011 Sep;17(9):1751–1754. https://doi.org/10.3201/eid1709.101931

Zowawi HM, Sartor AL, Sidjabat HE, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, Alfaresi M, Ibrahim E, Al-Jardani A, et al. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* isolates in the Gulf Cooperation Council States: dominance of OXA-23-type producers. J Clin Microbiol. 2015 Mar;53(3):896–903. https://doi.org/10.1128/JCM.02784-14