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

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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 blaOXA23-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.

**Key words:** 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 *blaNDM* gene and overexpression of the *blaOXA-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 *blaOXA-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...
Resistance associated gene
Mechanism
Resistance phenotypes

Aminoglycosides
16S rRNA methyltransferase genes
armA
Amikacin\(^1\), Gentamicin\(^1\), Tobramycin\(^1\)

Aminoglycoside modifying enzymes
aphA6, aadB, and aacC1

Carbapenems
Oxacillinas
blaOXA-23
Imipenem\(^1\), Meropenem\(^2\), Ceftazidime\(^3\), Cefotaxime\(^4\), Ceftriaxone\(^5\), Piperacillin-tazobactam\(^6\), Ampicillin-sulbactam\(^7\)

Fluoroquinolones
Quinolones Resistance Determining Region (QRDR)
gyrA gene mutation (Ser83Leu)
Ciprofloxacin\(^1\)

Sulfonamides
Dihydropteroate synthase
Sul2
Sulfamethoxazole-Trimethoprim\(^1\)

Tetracyclines
Tetracycline efflux MFS transporter
tetB
Doxycycline\(^4\)

\(^1\) MIC 1024 µg/ml, \(^2\) MIC 512 µg/ml, \(^3\) MIC 16 µg/ml, \(^4\) MIC 32 µg/ml, \(^5\) MIC 64 µg/ml, \(^6\) MIC 32 µg/ml, \(^7\) MIC 128/4 µg/ml, \(^8\) MIC 64/32 µg/ml, \(^9\) MIC 16 µg/ml, \(^10\) MIC 16/304 µg/ml, \(^11\) MIC 128 µg/ml

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

A 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 blaOXA-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., ISAba1, 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 (armA, rmtA, rmtB, rmtC, rmtD, and rmtE) 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 blaOXA-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 ISAba1 was found upstream to the blaOXA-51 and blaOXA-23 genes.

The concerns related to the possible threats of the blaOXA-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 extensive 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 blaOXA-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 blaOXA-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 blaOXA-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 (Punteener-Simmen et al. 2019). The carbapenem-resistant isolates in these studies were found to possess the intrinsic blaOXA-51 gene solely or accompanied by the acquired blaOXA-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 blaOXA-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.

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Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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