# Non-carbapenemase producing carbapenem-resistant *Klebsiella pneumoniae* isolated from the urinary tract of a dog

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## Abstract

**Objective**

Carbapenems are broad-spectrum β-lactams with excellent activity against multidrug-resistant (MDR) *Enterobacterales.* Unfortunately, resistance to carbapenems within this bacterial family, known as carbapenem-resistant *Enterobacterales* (CRE), occurs and challenges the ability to treat difficult MDR infections. Although the impact of carbapenem-resistance has been greatest in human medicine, reports in the veterinary literature are increasing especially as national veterinary antimicrobial resistance surveillance programs are now in place. In this brief communication, we report the isolation of a non-carbapenemase-producing, carbapenem-resistant *Klebsiella pneumoniae* from the urine of a dog, discuss the likely mechanism of resistance, and wider implications.

**Animal**

Canine

**Procedure**

Whole genome sequencing and phenotypic antimicrobial susceptibility testing was performed on a *K. pneumoniae* isolated from the urine of a dog.

**Results**

Antimicrobial susceptibility testing identified phenotypic resistance to imipenem and meropenem. Phenotypic detection of carbapenemase production was negative. Whole genome sequencing identified efflux pump genes associated with carbapenem resistance and point mutations in membrane porin genes. No carbapenemase gene was identified.

**Conclusion**

Phenotypic antimicrobial susceptibility testing identified the *K. pneumoniae* as a non-carbapenemase producing carbapenem-resistant organism with the proposed genotypic mechanism including alteration of efflux pumps and membrane porin activity and/or expression.

**Clinical significance**

Currently, there is limited use of carbapenem antimicrobial drugs in veterinary medicine, and practitioners may be unfamiliar or unaware of this type of resistance, its significance on routine antimicrobial susceptibility test reports, and implications for antimicrobial therapy and public health. Carbapenem-resistant *Enterobacterales* are infrequently isolated from companion animals; however, due to increasing adoption of advanced medical and surgical interventions, they may become more prevalent.

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## Résumé

***Klebsiella pneumoniae* résistante aux carbapénèmes non-productrice de carbapénémase isolée des voies urinaires d'un chien.**

**Objectif**

Les carbapénèmes sont des β-lactamines à large spectre avec une excellente activité contre les *Enterobacterales* multirésistantes (MDR). Malheureusement, la résistance aux carbapénèmes au sein de cette famille bactérienne, connue sous le nom d'*Enterobacterales* résistantes aux carbapénèmes (CRE), se produit et remet en question la capacité de traiter les infections MDR difficiles. Bien que l'impact de la résistance aux carbapénèmes ait été plus important en médecine humaine, les rapports dans la littérature vétérinaire se multiplient, d'autant plus que des programmes nationaux de surveillance de la résistance aux antimicrobiens vétérinaires sont désormais en place. Dans cette brève communication, nous rapportons l'isolement d'une *Klebsiella pneumoniae* non-productrice de carbapénémase et résistante aux carbapénèmes à partir de l'urine d'un chien, discutons du mécanisme probable de résistance et des implications plus larges.

**Animal**

Canin.

**Procédure**

Le séquençage du génome entier et les tests de sensibilité phénotypique aux antimicrobiens ont été effectués sur un isolat de *K. pneumoniae* provenant de l'urine d'un chien.

**Résultats**

Les tests de sensibilité aux antimicrobiens ont identifié une résistance phénotypique à l'imipénème et au méropénème. La détection phénotypique de production de carbapénèmase était négative. Le séquençage du génome entier a identifié des gènes de pompe à efflux associés à la résistance aux carbapénèmes et à des mutations ponctuelles dans les gènes des porines membranaires. Aucun gène de carbapénémase n'a été identifié.

**Conclusion**

Les tests de sensibilité phénotypique aux antimicrobiens ont identifié cet isolat de *K. pneumoniae* comme un organisme résistant aux carbapénèmes ne produisant pas de carbapénémase avec le mécanisme génotypique proposé, y compris l'altération des pompes à efflux et l'activité et/ou l'expression de porines membranaires.

**Signification clinique**

Actuellement, l'utilisation des médicaments antimicrobiens à base de carbapénème en médecine vétérinaire est limitée, et les praticiens peuvent ne pas être familiers ou ne pas être au fait de ce type de résistance, de son importance dans les rapports de routine sur les tests de sensibilité aux antimicrobiens et de ses implications pour la thérapie antimicrobienne et la santé publique. Les *Enterobacterales* résistantes aux carbapénèmes sont rarement isolées des animaux de compagnie; cependant, en raison de l'adoption croissante d'interventions médicales et chirurgicales avancées, elles peuvent devenir plus répandues.

(Traduit par Dr Serge Messier)


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A 13-year-old, 15.9 kg, spayed female mixed-breed dog with a 2-month history of chronic or recurrent urinary tract infection and a presumed underlying and uncontrolled endocrine disorder was presented for evaluation of a urinary tract infection. Urine obtained *via* cystocentesis was inoculated onto tryptic soy agar with 5% sheep blood and MacConkey agar (Remel, Lenexa, Kansas, USA) using 1 μL and 10 μL calibrated loops to allow for semi-quantitative analysis of growth. After overnight incubation at 35°C in room air, 3 unique bacterial colony morphologies were observed at greater than 100 000 colony forming units (CFU)/mL. Individual isolate identification was completed using Matrix-Assisted Laser-Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics Billerica, Massachusetts, USA). *Escherichia coli, Enterococcus faecium,* and *Klebsiella pneumoniae* were identified.

Antimicrobial susceptibility testing was done on the *K. pneumoniae* isolate using the broth microdilution method, the companion animal Gram-negative plate (COMPGN1F) and expanded Gram-negative nonfastidious plate (GN2F) and read using the automated ARIS system (Sensititre; ThermoFisher Scientific Waltham, Massachusetts, USA). The expanded plate provided additional cephalosporin, carbapenem, and monobactam agents not present in standard veterinary testing plates for more comprehensive assessment of resistance. Fosfomycin was individually tested (Etest; bioMérieux, Durham, North Carolina, USA). The modified carbapenem inactivation method (mCIM) test was performed using the Clinical and Laboratory Standards Institute (CLSI) method (1). Both *E. coli* and *E. faecium* were also tested, but no significant resistance was observed, and those results will not be discussed. Minimum inhibitory concentration values were interpreted using both veterinary and human CLSI criteria (1,2). Phenotypic resistance was noted for all β-lactam antimicrobial agents including penicillinase-labile penicillins, combination agents, cephalosporins, carbapenems, and

**Table 1.** Antimicrobial susceptibility test results.

| Class | Antimicrobial agent | MIC | Interpretive category |
|---|---|---|---|
| β-lactams | | | |
| Penicillinase-labile penicillins | Ampicillin | > 8 | R[C] |
| | Piperacillin | > 128 | R[H] |
| β-lactam combination agents | Ampicillin-sulbactam | > 32 | R[H] |
| | Amoxicillin-clavulanic acid | > 8 | R[C] |
| | Piperacillin-tazobactam | > 128 | R[H] |
| Cephems | Cephalothin | > 16 | R[H] |
| | Cefazolin | > 32 | R[C] |
| | Cefepime | > 16 | R[H] |
| | Cefotetan | > 32 | R[H] |
| | Cefovecin | > 8 | R[C] |
| | Cefpodoxime | > 8 | R[C] |
| | Ceftazidime | > 16 | R[C] |
| | Ceftriaxone | > 64 | R[H] |
| | Cefuroxime | > 16 | R[H] |
| Carbapenems | Imipenem | > 8 | R[H] |
| | Meropenem | 4 | R[H] |
| Monobactams | Aztreonam | > 32 | R[H] |
| Other | | | |
| Aminoglycosides | Amikacin | ≤ 4 | S[C] |
| | Gentamicin | 0.5 | S[C] |
| | Tobramycin | ≤ 4 | S[H] |
| Fluoroquinolones | Ciprofloxacin | ≤ 0.5 | S[H] |
| | Enrofloxacin | ≤ 0.12 | S[C] |
| | Gatifloxacin | ≤ 1 | S[H] |
| | Marbofloxacin | ≤ 0.12 | S[C] |
| | Orbifloxacin | ≤ 1 | S[C] |
| | Pradofloxacin | ≤ 0.25 | S[C] |
| Folate pathway antagonists | Trimethoprim-sulfamethoxazole | ≤ 0.5 | S[H] |
| Fosfomycins | Fosfomycin | > 256 | R[H] |
| Nitrofurans | Nitrofurantoin | 128 | R[H] |
| Phenicols | Chloramphenicol | 4 | S[H] |
| Tetracyclines | Doxycycline | 1 | S[H] |
| | Tetracycline | ≤ 4 | S[H] |

[C] Canine, Vet01S 5th ed.
[H] Human, M100, 31th ed.

monobactams. Measurement of the zone of inhibition following the mCIM procedure was 20 mm, indicating the *Klebsiella pneumoniae* did not hydrolyze the meropenem with a carbapenemase. Interestingly, both fosfomycin and nitrofurantoin were categorized as resistant (1), which are limited to use for urinary tract infections, and used primarily in humans. Results of testing are reported in Table 1, including CLSI criteria used.

To investigate the genetics underlying the phenotypic resistance observed, DNA was extracted from 10 isolated *K. pneumoniae* colonies using the DNeasy Blood and Tissue Kit (QIAGEN Sciences, Germantown, Maryland, USA) according to manufacturer's instructions. The DNA purity was measured by $A_{260}/A_{280}$ ratio on the Nanodrop (Thermo Fisher Scientific), according to manufacturer's instructions, resulting in an acceptable score of 1.86. The DNA concentration was measured using the Qubit dsDNA HS Assay Kit on a Qubit 4 Fluorometer (Thermo Fisher Scientific). The genomic library was prepared with 114 ng DNA using the Nextera Flex Library Preparation Kit (Illumina, San Diego, California, USA) according to the manufacturer's instructions. Sequencing was performed on the Illumina MiSeq platform, using v3, 2×250bp chemistry (Illumina). MiSeq data produced 98.6× coverage and an average read quality Q score for R1 and R2 of 35.74 and was considered of high quality for bacterial pathogens.

The MiSeq data were assembled using skesaMLST (3), and contigs were scanned for antimicrobial resistance genes using Staramr (Table 2). Virulence factor genes that could be associated with antimicrobial resistance were identified using Virulence Factors of Pathogenic Bacteria (VFPB) VFanalzyer (Table 2) (4,5). No carbapenemase gene was detected. A plasmid-mediated gene encoding a AmpC β-lactamase, $bla_{CMY-2}$, was present as well as the efflux pump (*acrAB*), which, in combination, are described mechanisms of resistance for CRE (6). Outer membrane porin genes *ompk35* and *ompk36* had 47 and 45 single nucleotide polymorphisms (SNPs), respectively, which have been associated with carbapenem resistance in combination with AmpC β-lactamase production (7). Changes in expression or activity for the efflux pump or porins were not confirmed for the *K. pneumoniae*. Antimicrobial resistance genes were also detected for fosfomycin *(fosA)* and fluoroquinolones *(oqxAB)*.

Whole genome sequencing revealed the likely resistance mechanism, a lesser known of 2 main mechanisms of

**Table 2.** Relevant antimicrobial resistance genes.

| Gene | Action | Drug class effected |
|---|---|---|
| $bla_{CMY-2}$ | β-lactamase | β-lactams |
| *fosA* | Fosfomycin resistance | Fosfomycin |
| *oqxA* | Multi-drug transporter | Multiple |
| *oqxB* | Multi-drug transporter | Multiple |
| *acrA* | Efflux pump: AcrAB | Multiple |
| *acrB* | Efflux pump: AcrAB | Multiple |

carbapenem-resistance described in *Enterobacterales.* The first was production of a carbapenemase that inactivated the carbapenem drug, referred to as carbapenemase (CP) CRE (8). The second results from a combination of multiple resistance mechanisms including production of an extended-spectrum β-lactamase with either increased expression of an efflux pump or decreased expression of an outer membrane porin, and denoted as non-CP CRE (8). In this case, the additive effect of multiple resistance mechanisms, plasmid-mediated AmpC activity in combination with efflux pump activity, produced the phenotypic *in vitro* profile (6). Other beta-lactam hydrolyzing enzymes, such as extended-spectrum beta-lactamases (ESBL) may produce a similar phenotype (8). Up to 70% of human CRE are non-CP CRE and lower MICs than CP-CREs (8).

Resistance to carbapenems through acquisition of carbapenemase genes are of greatest concern. These enzymes have varying ability to hydrolyze β-lactam agents, including penicillins, cephalosporins, and carbapenems (9). Carbapenemases are in Ambler classes A, B, and D based on amino acid homology and contain hundreds of genes (10). Not all have been determined to have clinical relevance; some may be clinically silent or have as yet undescribed significance (10). Carbapenemases have been identified from veterinary-origin isolates and include oxacillinase (OXA), New Delhi metallo-β-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), imipenemase (IMP), and Verona integrin-encoded metallo- β-lactamase (VIM) genes (11). Recently, an outbreak of *E. coli* carrying the NDM-5 carbapenemase gene was reported at a veterinary teaching hospital, highlighting the potential impact of CREs in veterinary medicine (12). Whereas non-CP-CRE are still of concern, CP-CRE are more feared due to dissemination of carbapenemase genes among various types of bacteria, which also leads to increased infection control difficulties and greater potential for wider spread in hospital settings (8). The significance of CP and non-CP CRE in veterinary species is unknown but presumed to be similar.

Carbapenems are used in human medicine for serious Gram-negative MDR infections, e.g., *Klebsiella pneumoniae,* in which resistance has developed to first- and second-tier antimicrobials. This is driven in large part through use of third-generation cephalosporins for Gram-negative infections, which are frequently used in veterinary medicine. One limitation in the use of carbapenems in veterinary species is the need for intravenous administration, generally necessitating hospitalization for the duration of treatment. Newer carbapenems, such as meropenem, are easier to administer, tend to have fewer side effects, and have a better Gram-negative spectrum than older members such as imipenem-cilastatin (11). Due to infrequent CRE infections and constraints in dosing, in rare instances carbapenems may be used to treat serious drug-resistant infections in companion animals; this is generally undertaken at tertiary care or teaching hospitals. Carbapenem use in agricultural animals is strongly discouraged, if not prohibited, depending on the country, and will not be discussed.

Interestingly, resistance was also observed for fosfomycin and nitrofurantoin, both common antimicrobial agents used for human urinary tract infections, but uncommonly used in veterinary medicine and not used in this case. Gene correlation was observed between *fosA* and phenotypic fosfomycin resistance, but for nitrofurantoin was likely due to efflux pump activity associated with *oqxAB.* No phenotypic resistance was observed for fluoroquinolone antimicrobial agents despite the presence of *oqxAB* genes, but the correlation between OqxAB, a multi-drug efflux pump, and resistance is poor, likely due to differences in expression level (13). These results supported the complementary approach of genotypic and phenotypic testing to identify the complete repertoire of antimicrobial resistance in bacteria.

The dog in question was not significantly ill, but rather had a very common clinical presentation to a primary care practice: an unresolved urinary tract infection. The patient had presumed underlying urinary dysfunction, highly suspected to be diabetes insipidus, and received multiple courses of antimicrobial agents in the 2 mo prior to the positive CRE culture. Confounding clinical significance, the culture was mixed with *Escherichia coli* and *Enterococcus faecium,* both common UTI pathogens. Re-collection of a urine sample was recommended due to concerns of sampling contamination, but this was not done. However, polymicrobial infection could also be plausible due to underlying disease. Regardless of the clinical uncertainty, asymptomatic bacteriuria, urinary tract infection, or contamination, the significance of the phenotypic resistance to imipenem and meropenem observed in the *K. pneumoniae* cannot be disputed (14). In this particular case, multiple antimicrobial drug options were available, and ultimately enrofloxacin and chloramphenicol were selected. Upon re-culture no bacteriuria was detected.

In general practice, the finding of a carbapenem-resistant bacteria may be missed or misunderstood by veterinarians due to its rarity unless alerted by the diagnostic laboratory. Routine reporting of carbapenem drugs results may be common in some laboratories but may be suppressed by others based on internal polices and/or antimicrobial stewardship programs. Thus, it is important for veterinarians to understand: i) what drugs represent significant resistance, and ii) how this is addressed in the laboratory being used. The most common carbapenem reported is likely to be imipenem, and very occasionally meropenem, and thus a quick scan of a report is easily done. If tiered reporting occurs and no carbapenem drug reported per laboratory antimicrobial stewardship protocols, then it is the responsibility of the laboratory to alert the clinician. Carbapenem-resistant *Enterobacterales* may also be reportable to the state or area human health authority and may come under investigation by public health personnel. An understanding of regional reporting requirements is highly recommended. Lastly, it is important to

note that clinical interpretive criteria for carbapenems are of human origin and are designed with human clinical outcome in mind. Therefore, if carbapenems are needed for a serious MDR Gram-negative infection in a veterinary species there is less confidence in the data predicting clinical outcome.

Although carbapenem resistance in Gram-negative bacteria is well-known and well-feared in human medicine, veterinary medicine has been spared significant impacts as yet. With advances in veterinary medicine, significant resistance in pathogens of veterinary origin will become a more common occurrence and therefore more education is needed. With the support of national AMR surveillance efforts allowing for genetic study of important veterinary pathogens we will have a greater understanding and be better prepared to face significant AMR resistance in the veterinary profession (15). CVJ

## References


1. CLSI. M100: Performance Standards for Antimicrobial Susceptibility Testing. 31st ed.: Clinical and Laboratory Standards Institute, 2021.
2. CLSI. VET01S: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 5th ed.: Clinical and Laboratory Standards Institute, 2020.
3. Souvorov A, Agarwala R, Lipman DJ. SKESA: Strategic k-mer extension for scrupulous assemblies. Genome Biol 2018;19:153.
4. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67: 2640–2644.
5. Carattoli A, Hasman H. PlasmidFinder and In silico pMLST: Identification and typing of plasmid replicons in whole-genome sequencing (WGS). Methods Mol Biol 2020;2075:285–294.
6. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev 2009;22: 161–182.
7. Arabaghian H, Salloum T, Alousi S, Panossian B, Araj GF, Tokajian S. Molecular characterization of carbapenem resistant *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* isolated from Lebanon. Sci Rep 2019;9:531.
8. Carbapenem-resistant *Enterobacterales* (CRE). Centers for Disease Control and Prevention NCfEaZIDN, Division of Healthcare Quality Promotion (DHQP): Center for Disease Control and Prevention. Available from: https://www.cdc.gov/hai/organisms/cre/index.html Last accessed April 21, 2022.
9. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev 2007;20:440–458.
10. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010;54:969–976.
11. Anderson REV, Boerlin P. Carbapenemase-producing *Enterobacteriaceae* in animals and methodologies for their detection. Can J Vet Res 2020; 84:3–17.
12. Cole SD, Peak L, Tyson GH, Reimschuessel R, Ceric O, Rankin SC. New Delhi metallo-β-lactamase-5-roducing *Escherichia coli* in companion animals, United States. Emerg Infect Dis 2020;26:381–383.
13. Li J, Zhang H, Ning J, et al. The nature and epidemiology of OqxAB, a multidrug efflux pump. Antimicrob Resist Infect Control 2019;8:44.
14. Holt KE, Wertheim H, Zadoks RN, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae,* an urgent threat to public health. Proc Natl Acad Sci USA 2015;112:E3574–3581.
15. Ceric O, Tyson GH, Goodman LB, et al. Enhancing the one health initiative by using whole genome sequencing to monitor antimicrobial resistance of animal pathogens: Vet-LIRN collaborative project with veterinary diagnostic laboratories in United States and Canada. BMC Vet Res 2019;15:130.