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Putative horizontal transfer of carbapenem resistance between *Klebsiella pneumoniae* and *Kluyvera ascorbata* during abdominal infection: A case report

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To the Editor—The emergence of KPC-producing *Enterobacter* has led to the development of serious infections related to high levels of mortality and morbidity worldwide.^{1,2} The rapid spread of KPCs is linked to multiple elements, such as plasmid-borne genes and the dissemination by international travelers; these bacteria are frequently multidrug resistant, causing untreatable infections.^{3,4} *Kluyvera* spp is a genus of gram-negative rods of the Enterobacteriaceae family.⁵ Although it is a commensal of the human gut microbiota,⁶ *Kluyvera* spp has the potential to cause septic shock, urinary tract infections, catheter-associated bloodstream infections, and abdominal infections.⁷ Here, we report a case of a plasmid-mediated horizontal transfer from a *Klebsiella pneumoniae* isolate to a *Kluyvera ascorbata* isolate during abdominal infection. The patient approved the data submission.

A 43-year-old male patient was admitted to the Hepatobiliary and Pancreatic Surgical Division from a hospital in the South Region of Brazil in October 2016. He was asymptomatic but had an incidental type I biliary cyst that was discovered during ultrasonography. Magnetic resonance imaging (MRI) with cholangiopancreatography was performed for adequate evaluation and showed an abnormal pancreatobiliary junction, as well. In November 2016, the patient underwent a cholecystectomy and total resection of the cyst, with closure of distal part of the main bile duct inside the pancreas, accompanied by Roux-en-Y hepaticojejunostomy to provide proper

biliary drainage. The pathology report showed no malignancy in surgical specimen. After 48 hours, the patient was evaluated with postoperative pancreatitis and signs of sepsis, therefore piperacillin/tazobactam treatment was started. The patient continued to present clinical deterioration and needed parenteral nutrition; he was consequently transferred to the intensive care unit (ICU). Blood cultures were negative and abdominal computerized tomography (CT) showed abdominal collections. CT-guided drainage of pancreatic fluid was performed and cultures were negative. Nevertheless, antimicrobial treatment with meropenem was started and continued for 14 days without resolution. A second CT-guided drainage procedure was performed at the end of December, and the bacteriological culture yielded a multisusceptible *Enterococcus faecalis*; a *Kluyvera ascorbata* resistant to ampicillin and second-generation cephalosporin but susceptible to carbapenem, and multidrug-resistant *Klebsiella pneumoniae*, including resistance to tigecycline and carbapenem. Antibiotics were adjusted to vancomycin, meropenem, and ertapenem plus polymyxin B with clinical improvement but without complete bacterial clearance. After 17 days, a third CT-guided drainage was performed, and the bacterial culture yielded 1 *K. ascorbata* isolate resistant to carbapenem. A fourth CT-guided drainage was performed after 15 days, and the culture yielded *K. ascorbata* susceptible to carbapenem and *K. pneumoniae* resistant to carbapenem. Antibiotic treatment was adjusted to polymyxin B, tigecycline, and sulfamethoxazol-trimetoprim, and the patient was evaluated with signs of controlled infection. After 14 days, the antibiotic treatment was suspended, and a final CT showed no signs of abdominal collections. The patient was discharged and was followed as an outpatient.

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Table 1. Antimicrobial Resistance Profile of the *Klebsiella pneumoniae* and *Kluyvera ascorbata* Pathogenic Isolates and the Transconjugants TKp and TKa

Antimicrobial Resistance	Microorganism				
	<i>E. coli</i> J53	<i>K. pneumoniae</i> KpOT1	<i>K. ascorbata</i> KaOT2	TKp	TKa
AMP	S	R	R	R	R
ASB	S	R	R	R	R
CFZ	S	R	R	R	R
CAZ	S	R	R	S	S
FEP	S	R	R	I	I
SXT	S	R	S	S	S
AK	S	I	S	S	S
GEN	S	I	S	S	S
CIP	S	R	S	S	S
MER	S	R	R	I	I
IMP	S	R	R	R	R
ERT	S	R	R	I	I
PTZ	S	R	R	I	I
MER MIC, g/mL	<0.0625	>32	>32	2	1
Resistance genes					
	...	<i>aph(3')-Ia</i> <i>aac(6')/Ib-cr</i> <i>bla_{KPC-2}</i> <i>bla_{SHV-11}</i> <i>bla_{CTX-M-15}</i> <i>bla_{OXA-1}</i> <i>oqxA</i> <i>oqxB</i> <i>fosA</i> <i>mph(A)</i> <i>catB4</i> <i>sul1</i> <i>tet(A)</i> <i>dfrA30</i>	<i>bla_{KPC-2}</i> <i>bla_{CTX-M-56}</i>	<i>bla_{KPC-2}</i>	<i>bla_{KPC-2}</i>


Note. AMP, ampicillin; ASB, ampicillin-sulbactam; CFZ, ceftazidime; CAZ, ceftazidime; FEP, cefepime; SXT, trimethoprim/sulfamethoxazole; AK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; MER, meropenem; IMP, imipenem; ERT, ertapenem; PTZ, piperacillin/tazobactam; MIC, minimum inhibitory concentration; *aph(3')-Ia*, aminoglycoside resistance; *aac(6')/Ib-cr*, fluoroquinolone and aminoglycoside resistance; *bla_{KPC-2}*, β-lactam resistance; *bla_{SHV-11}*, β-lactam resistance; *bla_{CTX-M-15}*, β-lactam resistance; *bla_{OXA-1}*, β-lactam resistance; *oqxA*, quinolone resistance; *oqxB*, quinolone resistance; *fosA*, fosfomycin resistance; *mph(A)*, macrolide resistance; *catB4*, phenicol resistance; *sul1*, sulphonamide resistance; *tet(A)*, tetracycline resistance; *dfrA30*, trimethoprim resistance; *bla_{CTX-M-56}*, β-lactam resistance.

In this case, 2 isolates were collected: 1 from the second CT, named *K. pneumoniae* KpOT1, and 1 from the third CT, named *K. ascorbata* KaOT2. Both were resistant to meropenem. They were forwarded to a molecular investigation of carbapenemase genes through conventional PCR, and both were *bla_{KPC-2}* positive. Conjugation experiments were performed using the azide-resistant *E. coli* J53 as the receptor strain. One transconjugant was obtained from each isolate; both presented an increase in the minimum inhibitory concentration (MIC)⁸ for meropenem, from <0.0625 µg/mL to 2 µg/mL (KpOT1 transconjugant) and 1 µg/mL, (KaOT2 transconjugant), confirming the transferability of the plasmids. The susceptibility profiles of KpOT1, KaOT2, and the transconjugants are shown in Table 1.

To better analyze the *bla_{KPC-2}* carrying plasmids, the whole genomes from both strains were sequenced using the Illumina

MiSeq platform (San Diego, CA). Detailed analyses indicated that the *bla_{KPC-2}* was located on an IncN plasmid. The carbapenemase resistance gene was flanked by the insertion sequences ISKpn7 and ISKpn6, located on a Tn4401 transposon, isoform b.⁹ The scaffold bearing the *bla_{KPC-2}* was 50,417 bp long, and no other resistance gene was found in this scaffold. The IncN plasmid was also found in the *K. ascorbata* genome, containing the same *bla_{KPC-2}* resistance gene and the same genome environment, indicating that a plasmid transference occurred between KpOT1 and KaOT2 when the patient was in the hospital unit. Further in silico analyses indicated that *K. pneumoniae* KpOT1 belonged to the sequence type ST437, one of the most prevalent sequence types among the KPC-producing *K. pneumoniae* and related to the clonal complex 258, which is distributed worldwide.^{10,11} Other resistance genes were identified in both isolates (Table 1). This whole-genome shotgun sequencing project has been deposited in the DDBJ/ENA/GenBank (accession no. RHF00000000 for *Klebsiella pneumoniae* OT1 and accession no. RHF00000000 for *Kluyvera ascorbata* OT2). The versions described in this article are versions RHF001000000 and RHF001000000.

This clinical case highlights the possibility of plasmid-mediated horizontal transfer between species during infections. Furthermore, KPC-2-producing *K. ascorbata* has only been isolated once, from a rectal swab in a surveillance study in Israel¹² and once in China from a patient's biliary drainage.¹³ We suggest that the carbapenem-susceptible *K. ascorbata* recovered in the fourth CT-guided drainage procedure could be related to a different clone or a heteroresistance event, but we cannot confirm this hypothesis. As far as we know, this is the first report of a KPC-2-carrying plasmid transference from a multidrug-resistant *Klebsiella pneumoniae* ST 437 to a *Kluyvera ascorbata* during abdominal infection.

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Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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Mycobacterium saskatchewanense strain associated with a chronic kidney disease patient in an Italian transplantation hospital and almost misdiagnosed as *Mycobacterium tuberculosis*

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To the Editor—During an evaluation for a solid organ transplant (SOT) procedure, we identified a rare, opportunistic nontuberculous (NTM) strain, *Mycobacterium saskatchewanense*. The patient, who suffered from chronic renal disease, was tested for several pathogens, *Mycobacterium tuberculosis* included. Transplantation procedures rely on immunosuppressive therapies, which increase the success of the surgery and the survival of the patient.¹ However, they also render the patients more susceptible to microbial infections, such as multidrug-resistant bacterial species like *Klebsiella pneumoniae*.^{2,3} Thus, patients require rigorous evaluations to confirm that they are not infected with pathogenic microorganisms that might compromise their health or lead to the rejection of the transplanted organ. One of the most opportunistic pathogens that can cause complications in SOT is *M. tuberculosis*, which infects up to 6.4% of transplant recipients in developed countries and up to 15% in areas where tuberculosis is highly endemic.⁴ Thus, exhaustive investigations, including the use of classical diagnostic microbiological and molecular assays are essential to ensuring that patients are fit for a SOT procedure or that they receive the correct antimicrobial therapy.

At the Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IRCCS-ISMETT), in Palermo, Italy, a 46-year-old man with hereditary chronic renal disease requiring dialysis for the previous 2 years was evaluated for renal transplantation. He had a positive interferon- γ release assay, suggesting prior exposure to *M. tuberculosis*. This patient had no previous history of tuberculosis infection, and his chest imaging did not show any signal of *M. tuberculosis* infection in the lungs. To confirm the presence of mycobacterial antigens in the blood, an ELISpot and T-SPOT.TB (Oxford Immunotec, Oxford, UK) were performed according to the manufacturer's instructions. Samples of sputum

and urine were also inoculated into liquid BBL mycobacteria growth indicator tubes (Becton Dickinson, Milan, Italy) according to the supplier's instructions to identify potential Mycobacteria. We obtained positive results for the presence of *M. tuberculosis* antigens in the blood and observed mycobacterial growth in the urine sample. Sputum samples were negative.

Because no *M. tuberculosis* was present in the patient's sputum and because this particular ELISpot has previously shown some limitations,⁵ we decided to extract the genomic material of this strain for sequencing. Total mycobacterial DNA was extracted using a QIAamp UCP Pathogen Mini Kit (Qiagen, Venlo, Netherlands), as specified by the kit's manual. Next-generation sequencing (NGS, full genome) and Sanger sequencing (16S rRNA gene, internal transcribed spacer (ITS1) 16S-23S and *hsp65*) were performed as published elsewhere.^{2,6} The sequences derived from both techniques showed a homology >99% with the publicly available sequences of previously identified NTM *M. saskatchewanense*, from the complex *M. simiae*,⁷ previously only reported in the United States⁸ and Canada.⁶ This particular NTM had been isolated from a bronchiectasis patient, both from sputum and from thoracentesis fluid, and was thought to have contributed to the deterioration of the patient.⁶ Genomic sequences of the *M. saskatchewanense* ISMETT strain were deposited in the NCBI public database (accession no. SRP149411).

The ELISpot was repeated 3 weeks later, yielding a negative result. Thus, and since we had confirmed that he was not infected with *M. tuberculosis*, the patient was not subjected to any antimycobacterial therapy. Because these interferon- γ release assays do not allow proper distinction between colonization and infection,⁹ we can only assume, due to its brevity, that the patient underwent a self-resolving episode of *M. saskatchewanense* colonization.

To the best of our knowledge, this is the first time this particular NTM has been detected outside North America. We assessed its antimicrobial sensitivity to several antibiotics. The minimal inhibitory concentrations (MIC) were determined using SlowMyco Sensititre plates (Thermo Fisher Scientific, Waltham, MA), according to the

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