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CASE REPORT

Cross-Infection of Solid Organ Transplant Recipients by a Multidrug-Resistant *Klebsiella pneumoniae* Isolate Producing the OXA-48 Carbapenemase, Likely Derived from a Multiorgan Donor

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We describe two cases of bacteremic infections caused by a multidrug-resistant *Klebsiella pneumoniae* isolate producing the OXA-48 carbapenemase that occurred in two solid organ transplant (liver and kidney) recipients, which was apparently transmitted with the allografts. This finding underscores the risk of donor-derived infections by multidrug-resistant Gram-negative pathogens in solid organ transplant recipients and emphasizes the need for rapid screening of organ donors for carriage of similar pathogens.

CASE REPORTS

Case 1. A 43-year-old patient with a history of chronic renal failure, HIV and hepatitis C virus (HCV) positivity, and drug addiction underwent a kidney transplantation at the University Hospital of Varese (northern Italy). Ampicillin-sulbactam was given for surgical prophylaxis and discontinued after 48 h. Six days after the transplantation procedure, the patient was febrile, and blood cultures revealed bacteremia caused by a Gram-negative rod. Empirical treatment with meropenem (1 g after dialysis) and gentamicin (240 mg in a single dose) was started. The isolate (HB1) was reported as *Klebsiella pneumoniae* producing extended-spectrum β-lactamase (ESBL), resistant to fluoroquinolones, expanded-spectrum cephalosporins, and ertapenem, but susceptible to meropenem, aminoglycosides, colistin, and tigecycline. Upon receipt of the susceptibility data, the treatment was de-escalated to meropenem only, in consideration of a delayed functional recovery of the transplanted kidney. Subsequent blood cultures yielded negative results, but meropenem treatment was continued since *K. pneumoniae* isolates with the same resistance profile as HB1 were repeatedly isolated from the surgical site, which eventually underwent dehiscence. The transplanted kidney did not exhibit functional recovery and was explanted 1 month after transplantation. Thereafter, the surgical site continued to yield *K. pneumoniae* with the same resistance profile as HB1. The patient was voluntarily discharged 1 week after allograft explantation, against medical opinion, and was lost at the follow-up. It should be noted that a *K. pneumoniae* isolate with the same resistance profile as HB1 (isolate CM1) had been isolated from the kidney preservation fluid at the transplantation center. This finding had been reported after the kidney transplantation procedure was completed but at that time was not considered to be significant.

Case 2. A 63-year-old patient with a history of hepatocarcinoma in HCV-related cirrhosis underwent liver transplant at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies of Palermo (southern Italy). The transplantation was carried out on the same day as that of case 1, and the organ was from the same donor. Ampicillin and cefotaxime were given for surgical prophylaxis and discontinued after 72 h. Four days after the transplantation procedure, the patient was febrile, and piperacillin-tazobactam (4.5 g three times a day [t.i.d.]) was started empirically in combination with gentamicin (300 mg) and vancomycin (1 g) given once. Bacteremia caused by a Gram-negative rod was detected in a central venous catheter-derived blood culture taken 5 days after transplantation, and colistin (1,000,000 U t.i.d.) was added in consideration of the knowledge that the organ donor was colonized by a multidrug-resistant (MDR) *Acinetobacter* isolate (described below). The isolate (GP1) was reported as *K. pneumoniae* with an MDR phenotype overall similar to that of HB1 and CM1. *K. pneumoniae* isolates with the same resistance profile were also obtained from subsequent blood cultures and from samples taken from the surgical wound and from the abdominal drainage fluid. Upon receipt of the susceptibility data, the treatment was shifted to meropenem (1 g t.i.d.) plus gentamicin (240 mg once a day) for 5 days. Meropenem was then replaced with ertapenem (1 g once a day), and antibiotic treatment was prolonged for one more week. The rationale for replacing meropenem with ertapenem at this time was not explained. Fever subsided, and infection was successfully treated. Two months after transplantation, the patient underwent a retrograde cholangiopancreatography (RCP) for treatment of an anastomotic biliary stenosis. The biliary aspirate yielded a *K. pneumoniae* isolate (GP2) with a similar resistance pattern to GP1, but no further

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TABLE 1 Antimicrobial susceptibilities of the four *K. pneumoniae* isolates studied in this work

Antibiotic(s)	MIC (mg/liter) for isolate[a]:			
	HB1	CM1	GP1	GP2
Imipenem	8 (I)	4 (I)	4 (I)	4 (I)
Meropenem	2 (S)	1 (S)	2 (S)	2 (S)
Ertapenem	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Ceftazidime	64 (R)	32 (R)	64 (R)	64 (R)
Cefotaxime	>32 (R)	>32 (R)	>32 (R)	>32 (R)
Cefepime	32 (R)	16 (R)	16 (R)	16 (R)
Amoxicillin-clavulanate	>128 (R)	>128 (R)	>128 (R)	>128 (R)
Piperacillin-tazobactam	>256 (R)	>256 (R)	>256 (R)	>256 (R)
Ampicillin-sulbactam	>32 (R)	>32 (R)	>32 (R)	>32 (R)
Gentamicin	1 (S)	1 (S)	1 (S)	1 (S)
Amikacin	4 (S)	4 (S)	8 (S)	4 (S)
Colistin	1 (S)	0.5 (S)	0.5 (S)	1 (S)
Tigecycline	1 (S)	1 (S)	1 (S)	1 (S)
Ciprofloxacin	>2 (R)	>2 (R)	>2 (R)	>2 (R)
Levofloxacin	>4 (R)	>4 (R)	>4 (R)	>4 (R)
Trimethoprim-sulfamethoxazole	>4/76 (R)	>4/76 (R)	>4/76 (R)	>4/76 (R)

[a] Susceptibility categories are given in parentheses: I, intermediate; S, susceptible; R, resistant.

antibiotic treatment was administered. Six months after transplantation, another RCP was carried out. On that occasion, no *K. pneumoniae* isolate was cultured from the biliary aspirate, which yielded one isolate each of *Escherichia coli*, *Proteus mirabilis*, and viridans group *Streptococcus*.

Organ donor. The organ donor was a 52-year-old subject from southern Italy, who died after cranial trauma and who (i) had a history of pulmonary tuberculosis (at 32 years of age) with negative results for the presence of acid-fast bacilli in respiratory secretions and no signs of active tuberculosis, (ii) had a record of promiscuous sexual behavior, (iii) tested negative for HbsAg, hepatitis B virus (HBV) DNA, anti-HCV antibodies, HCV RNA, anti-HIV antibodies or p24 antigen, and HIV RNA, and (iv) was colonized by a carbapenem-resistant *Acinetobacter* (CRA) isolate in the respiratory tract. Blood and urine cultures, taken on the same day of organ explantation, were negative. The liver and the kidney were harvested by the same surgical team and placed in different containers with preservation fluid. Altogether, the donor was considered at "increased" risk for infection transmission, according to the Italian and Council of Europe Guidelines (1), limiting the potential recipients to HIV-positive subjects or clinical emergencies. Due to the reported colonization by CRA of the donor, serial blood culturing was recommended in the organ recipients after transplantation, although the risk for transmission for this MDR pathogen was considered negligible.

Characterization of the *K. pneumoniae* isolates. The isolates HB1, CM1, GP1, and GP2 were available for further analysis. Identification as *K. pneumoniae* was confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Vitek-MS; bioMérieux, Marcy l'Etoile, France). MICs of the isolates were determined by reference broth microdilution (2), and results were interpreted according to the EUCAST breakpoints (EUCAST breakpoint tables for interpretation of MICs and zone diameters, version 4.0, 2014; http://www.eucast.org). The resistance profiles of the isolates were very similar, including β-lactamase inhibitor combinations, expanded-spectrum cephalosporins, ertapenem, fluoroquinolones, and trimethoprim-sulfamethoxazole. The isolates were intermediate to imipenem but remained susceptible to meropenem (although MIC values were close to the susceptibility breakpoint), aminoglycoside, colistin, and tigecycline (Table 1). All isolates tested positive for ESBL production by disk synergy testing and for carbapenemase production by modified Hodge test (3, 4), while neither EDTA nor boronate showed synergistic activity with meropenem in disk synergy testing (5).

The presence of acquired β-lactamase genes was determined by a DNA microarray followed by PCR and sequencing, as described previously (6). This analysis revealed the presence of the $bla_{\text{OXA-48}}$ carbapenemase gene, the $bla_{\text{CTX-M-15}}$ ESBL gene, and the $bla_{\text{TEM-1}}$ broad-spectrum β-lactamase gene in all isolates.

Characterization of the four isolates by pulsed-field gel electrophoresis (PFGE) profiling of genomic DNA digested with XbaI (7) revealed an identical profile (Fig. 1). Multilocus sequence typing (MLST) (8) of the four isolates revealed that all belonged in sequence type 16, a clonal lineage reported in several countries (e.g., Canada, Brazil, and Spain) and found to be associated with clinically relevant β-lactamases, including NDM-1, KPC-2, and OXA-48 (9–11).

Infections caused by multidrug-resistant (MDR) bacteria are a major emerging challenge. The problem is particularly serious with carbapenemase-producing *Enterobacteriaceae* (CPE), which usually exhibit extended-drug-resistance phenotypes and remain susceptible to only a few antibiotics (12, 13).

Solid organ transplant (SOT) recipients are at increased risk of developing infections caused by MDR bacteria due to a number of risk factors inherent to these patients, including immunosuppression, invasive procedures, and prolonged exposure to the hospital

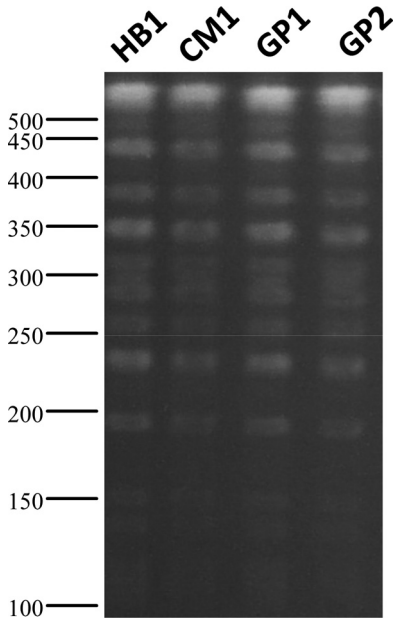

FIG 1 PFGE profiles of XbaI-digested genomic DNAs showing genomic relatedness among the *K. pneumoniae* isolates. DNA size standards are indicated (in kb) on the left.

environment and antibiotic treatments (14–16). These infections are typically caused by pathogens acquired from the hospital environment or already present as colonizers in the transplant recipient (14, 17, 18). However, cross-transmission of MDR bacteria is also possible from donors infected or colonized by MDR bacteria. In fact, few reports have dealt with cross-transmission of CPE from organ donors to transplant recipients (18–20), but the risk of similar events is expected to increase following the ongoing global spread of CPE (12, 13).

The hypothesis that the infections that occurred in the two transplant recipients were derived from cross-transmission of the *K. pneumoniae* strain via the transplanted organs is strongly supported by the timing of the infections, by the identity of the *K. pneumoniae* isolates, including that found in the kidney perfusion fluid, and by the fact that OXA-48-producing *K. pneumoniae* isolates were very uncommon in Italy at the time of the infections (mid-2011) (21). The donor was most likely the source of the OXA-48-producing *K. pneumoniae* isolate. However, since no *K. pneumoniae* isolate was reported from the cultured donor samples, contamination of the organs at the time of harvesting cannot be excluded. It should be noted that although no specific information was available, the donor had likely been given a broad-spectrum antibiotic regimen, which might have contributed to reducing the detection sensitivity of the cultures for the *K. pneumoniae* strain.

To our knowledge, this represents the first report of likely donor-derived infections by an OXA-48-producing *K. pneumoniae* isolate in SOT recipients. In both transplanted patients, the MDR pathogen caused a bacteremic infection associated with infection of the surgical site. The use of an active carbapenem in combination with at least one other active agent was successful in controlling bacteremia, but in one case, it could not control the infection at the surgical site.

Similar findings raised some questions about (i) the adequacy of donor screening protocols for assessment of the risk of transmission of CPE, which is expected to increase due to the recent epidemiological diffusion of CPE, and (ii) the suitability of organs from donors infected or colonized by CPE.

Concerning the first point, investigation of donors for CPE carriage by suitable approaches (e.g., rectal swabbing) would seem mandatory, especially in areas where CPE are endemic. In fact, also following the present observation, the Italian guidelines for evaluation of potential organ donors have been updated to acknowledge the risk of cross-transmission of MDR or panresistant bacteria to recipients and specifically require that donors be screened for these pathogens by suitable microbiological techniques. In this perspective, since transplantation must be done expeditiously to ensure organ viability, while the results of bacteriological cultures collected at the time of organ procurement may become available only after the transplant has been performed, molecular biology can be faster and more sensitive than conventional culture for detection of CPE from organ donors and should be included as an additional approach in the laboratory protocols.

Concerning the second point, it has been suggested that organs from donors colonized with CPE may be considered for transplantation under well-defined conditions (18, 19). However, the risk of cross-infections in these cases appears to be high, and the potential consequences should be carefully considered.

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REFERENCES

1. **Nanni Costa A, Grossi P, Gianelli Castiglione A, Grigioni WF.** 2008. Quality and safety in the Italian donor evaluation process. Transplantation **85:**S52–S56. http://dx.doi.org/10.1097/TP.0b013e31816c2f05.
2. **Clinical and Laboratory Standards Institute.** 2012. Methods for dilution antimicrobial susceptibility. Tests for bacteria that grow aerobically; Approved standards, 9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Reference deleted.
4. **Clinical and Laboratory Standards Institute.** 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. Approved standard M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Giske CG, Gezelius L, Samuelsen O, Warner M, Sundsfjord A, Woodford N.** 2011. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin. Microbiol. Infect. **17:**552–556. http://dx.doi.org/10.1111/j.1469-0691.2010.03294.x.
6. **D'Andrea MM, Venturelli C, Giani T, Arena F, Conte V, Bresciani P, Rumpianesi F, Pantosti A, Narni F, Rossolini GM.** 2011. Persistent carriage and infection by multidrug-resistant *Escherichia coli* ST405 producing NDM-1 carbapenemase: report on the first Italian cases. J. Clin. Microbiol. **49:**2755–2758. http://dx.doi.org/10.1128/JCM.00016-11.
7. **Yuan M, Aucken H, Hall LM, Pitt TL, Livermore DM.** 1998. Epidemiological typing of *Klebsiellae* with extended-spectrum beta-lactamases from European intensive care units. J. Antimicrob. Chemother. **41:**527–539. http://dx.doi.org/10.1093/jac/41.5.527.
8. **Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol. **43:**4178–4182. http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005.
9. **Oteo J, Hernández JM, Espasa M, Fleites A, Sáez D, Bautista V, Pérez-Vázquez M, Fernández-García MD, Delgado-Iribarren A, Sánchez-Romero I, García-Picazo L, Miguel MD, Solís S, Aznar E, Trujillo G, Mediavilla C, Fontanals D, Rojo S, Vindel A, Campos J.** 2013. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. J. Antimicrob. Chemother. **68:**317–321. http://dx.doi.org/10.1093/jac/dks383.
10. **Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA.** 2011. New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada. Emerg. Infect. Dis. **17:**103–106. http://dx.doi.org/10.3201/eid1701.101358.
11. **Seki LM, Pereira PS, de Souza MDPAH, de Conceição M, Marques SEA, Porto CO, Colnago EML, de Alves C, Gomes FMD, Assef APDAC, Samuelsen Ø, Asensi MD.** 2011. Molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* isolates in Brazil: the predominance of sequence type 437. Diagn. Microbiol. Infect. Dis. **70:**274–277. http://dx.doi.org/10.1016/j.diagmicrobio.2011.01.006.
12. **Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P, the European Network on Carbapenemases.** 2012. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin. Microbiol. Infect. **18:**413–431. http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x.
13. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **17:**1791–1798. http://dx.doi.org/10.3201/eid1710.110655.
14. **Fishman JA, Greenwald MA, Kuehnert MJ.** 2007. Enhancing transplant safety: a new era in the microbiologic evaluation of organ donors? Am. J. Transplant. **7:**2652–2654. http://dx.doi.org/10.1111/j.1600-6143.2007.02023.x.
15. **Lübbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, Bartels M, Kaisers UX.** 2014. Colonization of liver transplant recipients with KPC-producing *Klebsiella pneumoniae* is associated with high infection rates

and excess mortality: a case-control analysis. Infection **42:**309–316. http://dx.doi.org/10.1007/s15010-013-0547-3.

16. **van Duin D, Van Delden C.** 2013. Multidrug-resistant Gram-negative bacteria infections in solid organ transplantation. Am. J. Transplant. **13:** 31–41. http://dx.doi.org/10.1111/ajt.12096.
17. **Kalpoe JS, Sonnenberg E, Factor SH, del Rio Martin J, Schiano T, Patel G, Huprikar S.** 2012. Mortality associated with carbapenem-resistant *Klebsiella pneumoniae* infections in liver transplant recipients. Liver Transpl. **18:**468–474. http://dx.doi.org/10.1002/lt.23374.
18. **Ariza-Heredia EJ, Patel R, Blumberg EA, Walker RC, Lewis R, Evans J, Sankar A, Willliams MD, Rogers J, Milano C, Razonable RR.** 2012. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. Transpl. Infect. Dis. **14:** 229–236. http://dx.doi.org/10.1111/j.1399-3062.2012.00742.x.
19. **Goldberg E, Bishara J, Lev S, Singer P, Cohen J.** 2012. Organ transplantation from a donor colonized with a multidrug-resistant organism: a case report. Transpl. Infect. Dis. **14:**296–299. http://dx.doi.org/10.1111/j.1399-3062.2011.00697.x.
20. **Watkins AC, Vedula GV, Horan J, Dellicarpini K, Pak S-W, Daly T, Samstein B, Kato T, Emond JC, Guarrera JV.** 2012. The deceased organ donor with an 'open abdomen': proceed with caution. Transpl. Infect. Dis. **14:**311–315. http://dx.doi.org/10.1111/j.1399-3062.2011.00712.x.
21. **Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, Survey Participants AMCLI-CRE, Pantosti A, Pagani L, Luzzaro F, Rossolini GM.** 2013. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. Euro Surveill. **18:**pii=20489. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20489.